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CONTENTS

Continued on back cover

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Further studies on the genetic divergence of multivoltine silkworm (*Bombyx mori* L.) genotypes based on economic characters

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ABSTRACT: Genetic divergence among 13 evolved and 9 geographical races of multivoltine silkworm were estimated on the basis of 5 important economic characters. The test of significance for multiple measurements using '*V*' statistics (1982.77), which utilized Wilk's criterion, confirmed significant differences among the genotypes. D^2 values computed for all possible 231 pairs ranged from 0.136 to 51.173. All the genotypes were grouped in six clusters based on Ward's minimum variance. The results suggest that there is no relation between geographical diversity and genetic diversity; besides the cocoon shape plays a vital role in the clustering. The optimum divergence obtained in cluster V and VI along with highest mean values and positive correlation for the selected attributes indicate that these races have the genetic potential to create maximum heterosis. Among the 22 races studied, Pure Mysore race is very unique and characteristic of low silk yield compared to many other races, suggesting that more productive multivoltine races should be popularized for improving the silk yield in India.

KEYWORDS: Genetic divergence, economic characters, multivoltine silkworm races

INTRODUCTION

In India, sericulture is mainly dependent on multivoltine breeds, which have characteristically low silk productivity and poor silk quality. In the process of genetic improvement, a number of races/strains were developed, which constitute the basis for future silkworm breeding programme; whose maintenance and utilization warrant proper classification and listing (Chatterjee and Datta, 1992). Mahalanobis D^2 statistics is a powerful tool for discerning nature and magnitude of genetic divergence among the genotypes, based on actual expression of multiple characters and it is essential to ascertain the level of parental divergence when an initial choice of parents

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has to be made to obtain heterotic hybrid (Jolly *et al.*, 1989). Inclusion of more diverse parents (within a limit) in hybridization is believed to increase the chances of obtaining stronger heterosis in hybrids (Joshi and Dhawan, 1966; Anand and Murty, 1968). In this line, a few studies were earlier made using quite a few silkworm races to classify them based on the quantitative and qualitative traits (Rao *et al.*, 1989; Chatterjee *et al.*, 1993; Ramamohana Rao and Nakada, 1998). The present investigation was undertaken using a sizable number of silkworm races to evaluate the genetic divergence among the selected geographical races and evolved breeds of 22 multivoltine silkworm on the basis of important economic characters.

MATERIALS AND METHODS

Twenty two silkworm genotypes comprised of 9 geographical races and 13 evolved breeds of multivoltine were used in the present study (Table 1). These genotypes are characterised and maintained in the germplasm bank of the Central Sericultural Germplasm Resources Centre, Hosur, Tamil Nadu (Altitude: 942 MSL; Latitude: 12°45′N; Longitude: 77°51′E; Temperature–Maximum: 27–36 °C, Minimum: 14–19 °C; Relative humidity–Maximum: 56–90%, Minimum: 37–59% and Annual average rain fall: 800–1000 mm). The rearing was conducted in completely randomized block design with two replications of 350 larvae each after third moult. The standard rearing techniques recommended by Krishnaswamy (1978) were followed. The data collected from six crops representing different seasons and conducted during 1996–2000 were considered. To ascertain the genetic divergence among the selected genotypes, the observation recorded on economic characters such as weight of 10 grown larvae (g), single cocoon weight (g), single shell weight (g), cocoon yield/10 000 larvae by weight (Kg) and pupation rate (%) were subjected to analysis of genetic divergence using Mahalanobis D^2 along with correlation analysis as per Singh and Chaudhary (1985) using the computer packages developed by Indostat Service Pvt. Ltd, Hyderabad, India.

RESULTS AND DISCUSSION

The test of significance for multiple measurements using '*V*' statistics (1982.77), which utilized Wilk's criterion, confirmed significant differences among these genotypes and suggested the adequacy of continuing of D^2 analysis.

D^2 values computed for all possible 231 pairs ranged from 0.136 (between NK4 and LMO) to 51.173 (between Pure Mysore and MHMP (Y)), where NK4 and Pure Mysore are geographical races and LMO and MHMP (Y) are evolved breeds. All the genotypes were grouped in six clusters based on Ward's minimum variance (Table 2). Cluster I containing 3 genotypes, *viz.*, C. nichi, Daizo and ZPN (SL), where C. nichi and Daizo are geographical races; C. nichi is considered to be of Japanese origin (shi × nichi) and Daizo is a very old silkworm race of China which were widely exploited in the early years. C. nichi is a peanut shaped cocoon race while others are spindle shaped. ZPN (SL) is sex limited breed in larval markings. Only one genotype, Pure

TABLE 1. Name, origin and cocoon characters of 22 multivoltine silkworm genotypes

Sl. No.	Name of the breed	Origin	Cocoon colour	Cocoon shape
	Geographical races			
1	Pure Mysore	KAR	Greenish yellow	Oval
2	Sarupat	ASM	Creamy white	SA
3	Moria	ASM	Creamy white	SA
4	C. nichi	JAP	White	Constricted
5	Raj	BNG	Creamy white	Spindle
6	Nistari	WBL	Golden yellow	Spindle
7	NK4	THI	Yellow	SA
8	Cambodg	CAM	Yellow	SA
9	Daizo	CHI	Greenish yellow	Spindle
	Evolved breeds			
10	Hosa Mysore	KAR	Greenish yellow	Elongated oval
11	Kolar Gold	KAR	White	Oval
12	Kollegal Jawan	KAR	White	Oval
13	ZPN(SL)	WBL	Creamy white	Spindle
14	CB5	WBL	Golden yellow	Oval
15	AP12	KAR	Greenish yellow	Elongated oval
16	A13	KAR	Greenish yellow	Elongated oval
17	MW13	KAR	White	Oval
18	MHMP (Y)	KAR	Greenish yellow	Elongated oval
19	P4D3	KAR	Greenish yellow	Elongated oval
20	LMO	WBL	Golden yellow	Oval
21	MY1 (SL)	KAR	LGY	Elongated oval
22	PM (SL)	KAR	LGY	Elongated oval

KAR = Karnataka, ASM = Assam, WBL = West Bengal, BNG = Bangladesh, THI = Thailand, CAM = Cambodia, JAP = Japan, CHI = China, LGY = Light greenish yellow, SA = Spindle attenuated.

Mysore, was isolated in cluster II which indicate its unique characteristic feature of low productivity; the race was considered to be brought from China during the regime of Tippu Sultan (200 years back) are widely exploited in south India for silk production. The storage protein studies of 11 multivoltine silkworm accession and clustering based on Ward's minimum variance analysis also indicate that the Pure Mysore race falls in a distinct group (Somasundaram *et al.*, 2002). Cluster III accommodated 4 genotypes *viz.*, Raj, Nistari, Cambodg and Sarupat were all of different geographical races and all of them produce spindle shaped cocoon showing their close affinity, which suggest that there is no relation between geographical diversity and genetic diversity. This findings corroborates earlier report of Jolly *et al.* (1989).

Cluster IV included four genotypes, *viz.*, NK4, LMO, Moria and CB5 in which NK4 and Moria are geographical races and LMO and CB5 are evolved breeds. This indicates that the breeds from diverse eco-geographical origin are not always related

TABLE 2. Clustering pattern of 22 multivoltine silkworm genotypes on the basis of genetic divergence

Cluster number	Number of genotypes	Genotypes included
I	3	C. nichi, Daizo, ZPN (SL)
II	1	Pure Mysore
III	4	Raj, Nistari, Cambodg, Sarupat
IV	4	NK4, LMO, MORIA, CB5
V	5	Hosa Mysore, P4D3, Kollegal Jawan, A13, MHMP (Y)
VI	5	Kolar Gold, MW13, PM (SL), MY1 (SL), AP12

TABLE 3. Average inter and intra cluster D^2 values among six clusters in multivoltine silkworm genotypes

Clusters	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Cluster I	**1.201**	3.362	2.330	3.444	5.307	4.070
Cluster II		**0.000**	4.056	5.641	6.584	5.444
Cluster III			**0.858**	1.760	3.189	2.618
Cluster IV				**0.718**	2.485	2.641
Cluster V					**0.939**	1.662
Cluster VI						**1.305**

to genetic diversity and this result confirmed the earlier findings of Ramamohana Rao and Nakada (1998). The results also suggest that the cocoon shape plays a vital role in the clustering, since most of the spindle shaped cocoon producing races are clustered together. This further implies that there might be relation between cocoon shape and genetic diversity. Cluster V and VI included five genotypes which were all evolved breeds; all the races have similar cocoon colour (greenish yellow) and most of them have elongated oval cocoon shape (Table 2). The inclusion of some silkworm breeds of the same origin in different clusters clearly indicates presence of considerable genetic diversity within the same group of breeds.

Intra-cluster value was minimum (0) where only one genotype (Pure Mysore) represented the cluster II. Highest value (1.305) was obtained in cluster IV, which included 5 genotypes followed by cluster I (1.201) and cluster V (0.939). Cluster II has shown the highest inter cluster distance with cluster V (6.584) followed with cluster IV (5.641), whereas the minimum inter cluster distance was recorded between cluster V and cluster VI (1.662) (Table 3). The results further suggest that the breeds derived from the same source were included in the same cluster showing a close affinity between advanced sister lines and also suggested the influence of female percentage.

While considering the genetic diversity for selection of genotypes to be included in the improvement programme, cluster means of selected attributes indicates, cluster V and VI expressed highest mean values for all the five characters (Table 4) and most of

TABLE 4. Cluster mean of five characters in multivoltine silkworm genotypes

Clusters	Wt. of 10 grown larvae (g)	Single cocoon Wt. (g)	Single shell Wt. (g)	Cocoon yield/10 000 larvae by Wt. (Kg)	Pupation rate (%)
Cluster I	20.975	1.041	0.136	8.245	81.993
Cluster II	18.756	1.076	0.149	7.771	71.536
Cluster III	23.769	1.184	0.171	9.642	81.943
Cluster IV	24.966	1.234	0.179	10.525	86.284
Cluster V	28.539	1.399	0.218	11.256	83.524
Cluster VI	27.081	1.363	0.208	10.869	79.877

TABLE 5. Correlation matrix between variables of multivoltine silkworm genotypes

Variables	WTG	CWT	SWT	YWT	PUPA
WTG	—	0.943**	0.896**	0.943**	0.327
CWT		—	0.967**	0.941**	0.151
SWT			—	0.906**	0.102
YWT				—	0.415
PUPA					—

WTG = Wt. of 10 grown larvae (g), CWT = single cocoon wt. (g), SWT = single shell wt. (g), YWT = cocoon yield/10 000 larvae by wt. (Kg), PUPA = pupation rate%.
**Significance at $P < 0.01$.

the genotypes were popularized in the field of tropical sericulture. Correlation analysis also revealed that all the attributes were positively correlated with high significance (Table 5). The positive correlation between these traits suggests the scope for using one set of variables as selection marker to control expression of other correlated variables (Chatterjee *et al.*, 1993).

Arunachalam *et al.* (1984) emphasized that there is an optimum level of genetic diversity between parents to obtain heterosis in F_1 generation and it may not be logical to advocate the use of extreme divergence parents to obtain heterotic combination. Hence the present investigation suggests, to create maximum possibility of getting transgressive segregants of practical utility emphasis should be given to include the genotypes of cluster V and VI to recover potential transgressive segregants for selected traits from crosses involving divergent parents.

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Evaluation of insects associated with the Bambara groundnut, *Vigna subterranea* (L) Verde, relative to planting date

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ABSTRACT: Insect species densities were determined on a local variety of the Bambara groundnut, *Vigna subterranea* (L) Verde in order to ascertain if planting date had any effect on the species diversity of pest and beneficial insects. The crop was cultivated at Luyengo in Swaziland, on five planting dates at three weekly intervals. Sowing date of the crop had no significant effect on the total species diversity of pests and benefical insects. However, pest species occurring during the first month after sowing. Insect damage on plants planted early in the season, treament 1, was due to feeding on foliage and flowers by phytophagous pests, e.g. lepidopteran larvae and weevils. On the other hand, late-planted crops, i.e. treatment 5, were heavily infested with aphids and had no damage due to feeding by phytophagous insects. This indicated that the time of planting has an effect on the fauna to which the plants are exposed. Identification of the various species observed as well as their activity in Bambara groundnut would contribute towards the formulation of informed guidelines for its cultivation for the resource poor farmers who depend on this crop.

KEYWORDS: Bambara groundnut, *Vigna subterranea*, planting date, Swaziland

INTRODUCTION

The Bambara groundnut, *Vigna subterranea* (L) Verde, is an underutilised leguminous crop indigenous to Africa. Literature indicates that the crop is a relatively well-balanced food, nutritionally superior to other legumes, tolerant to drought and low soil fertility. It is easily cultivated with little demand on the soil, if any (Doku, 1996). In Swaziland, Bambara groundnut is grown primarily as a subsistence crop, usually by resource poor farmers. The crop is important to the farmers as a dietary food as well as a source of income. Its major advantage to the farmers is its versatility, i.e. it is able to provide a good yield on marginal soil too poor to support other crops and supposedly resistant to a range of pests and diseases. However, despite its virtues, the

Bambara groundnut is one of Africa's most underutilised crops, i.e. ..'it is not fully exploited in terms of its potential use as a food or non-food crop' (Azam-Ali, 1996). One consequence of its underutilisation is the limited amount of research carried out on the crop (Azam-Ali, 1996). This is true in Swaziland, where only baseline research has only been recently initiated on the crop (Sesay *et al.*, 1999).

The insect fauna occurring in a crop system is an essential component in the determination of the crop's yield. Pests and diseases associated with the Bambara groundnut have been overlooked, largely due to the pest/disease free attitude towards the crop. Various studies have however, indicated that the crop is susceptible to some attack by a variety of pests. Surveys performed in Botswana and Sierra Leone indicated that pests on Bambara groundnut range from vertebrates, e.g. rodents and rabbits to a host of insects, e.g. termites, crickets, ants and grasshoppers. Post-harvest pests are mainly the weevils or bruchids (Brink *et al.*, 1996, Sesay *et al.*, 1996). A local farmer survey indicated that the main insects pests associated with this crop were perceived to be the aphids and to a lesser extent, termites, ants and Lepidopteran larvae.

This study was carried out to make a baseline assessment of pest and beneficial insects associated with the crop in Swaziland. The survey was also aimed at assessing if the species density on the Bambara groundnut is influenced by the planting date.

MATERIALS AND METHODS

The experiment was carried out on the Crop Production Research Farm at the University of Swaziland (Luyengo campus). A local Bambara groundnut genotype was planted on five dates (treatments) at three weekly intervals from 13 October 1999 to 5 January 2000. The treatments were arranged in a completely randomised block design, each with four replicates. Each plot had eight planting rows, 0.60 m apart and 6 m long with interplant distances at 0.15 m. No pesticides or herbicides were used during the period of observation, with only cultural methods used for crop maintenance, e.g. weeding, earthing up.

Survey methods utilised were those of pitfall trapping and visual observations. Putting a 250 ml plastic cup into a 0.20 m PVC pipes made pitfall traps, while PVC gutters cut in 0.20 m lengths were used as protective covers. The sides of the gutters were cut in order to enable movement of epigaeic fauna in all directions. Pitfall traps were sunk at 7 days after sowing for all treatments and were serviced every fortnight or after heavy rains.

Within each plot, 4 pitfall traps were randomly placed in rows between the plants. Each treatment (planting date) thus had 16 traps, making a total of 80 pitfall traps for the whole experiment. In order to minimise disturbance of the surroundings, the PVC pipes remained in the same position, with only the cups being changed. Antifreeze (ethylene glycol) diluted with distilled water (1 : 3) was used as a collecting fluid and preservative. Catch from each cup was sorted by running water through a sieve tower (three sieves with 2 mm, 1 mm and 500 μm apertures stacked in decreasing aperture size) and examining all the sieves against a white tray.

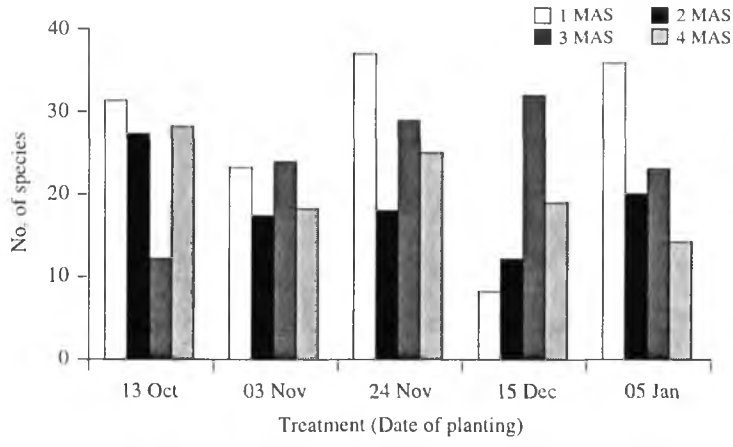

FIGURE 1. Species density for each treatment, at the indicated months after sowing (MAS). Plants harvested four months after sowing.

During the servicing of the traps, visual observations were also made on ten randomly selected plants within each plot. The presence of insects, as well as damage due to their feeding on leaves and stems, were noted. Specimens were identified using reference material while some were sent to the Transvaal museum.

Insect species diversity, i.e. the number of species, was determined by recording the presence or absence of each species. Data was recorded for each plot and each planting date at monthly intervals until harvesting and analysed using the SPSS package.

RESULTS

Overall species densities

Preliminary analyses indicated that the total species diversity was not significantly different between the replicates for all the planting dates ($P = 0.6647$). Data were therefore combined for each of the planting dates. A total of 89 species were collected during the period although identification for most of these was possible only up to the family level. Ten insect orders were collected, with the Coleoptera in the majority (Table 1) in terms of species diversity.

There were no significant differences in the overall species densities (ANOVA, $P = 0.7124$) between the five planting dates. With the exception of the plants sown in December, the plants had the highest species density during the first four weeks after sowing (Fig. 1). The lower species densities during this period observed in plants sown in December (treatment 4) may have been due to the unusually heavy rains received during that month, which would undoubtedly affect insect activity. Analyses, however, indicated that the differences in species densities relative to the age of the plant were not significant ($P = 0.3535$).

TABLE 1. Variety of insect families collected from all treatment groups and time of occurrence during the period of growth

Family	Species	Time of occurrence of insects (month after planting) Planting date				
		13 Oct.	03 Nov.	24 Nov.	15 Dec.	05 Jan.
Pests of seeds/pods/ Roots						
Termitidae	*Macrotermes* sp.	1–4*	1–4	1–4	2–3	2–3
Aphididae	*Aphis craccivora*	1	2–3	2–3	1, 4	1
Pests of leaves/ flowers/stems						
Curculionidae	*Lixus, Protostrophus, Blosyrinus* spp.	1–4	1–4	1–4	1–4	1–4
Melyridae	*Astylus atromaculatus*			4		
Tenebrionidae	*Gonocephalum*, 1 unidentified sp.	1–4	1–4	1–4	1–4	1–4
Lepidoptera	*Helicorvepa armigera, Protostrophus, Spodoptera* sp.	1, 4	1, 4	1–4	1, 4	2–3
Acrididae	2 species	1–4	1–3	1, 4	1, 4	4
Grylliade	1 species	1–4	4	4	4	4
Pseudococcidae	1 species	-	-	2–3	2–3	2–3
Chrysomelidae	8 species	1–4	1–4	1–4	1–4	1–4
Bruchidae	1 species	-	4	4	4	4
Cydnidae	1 species	1–4	1–4	1–4	1–4	1–3
Staphylinidae	3 species	1, 4	2–3	1–4	1	1–3
Reduviidae	1 species	1–3	4	4	4	4
Beneficial insects						
Cicindelidae	Dromica sp.	1, 4	1	2–3	2–3	
Coccinellidae	*Hippodamia variegata, Cheilomenes lunata, Scymnus* sp.	1–4	1–3	1–4	1–4	1–4
Carabidae	15 species	1–4	1–4	1–4	1–4	1–4
Apidae	1 species	1	1	1 -	-	
Anthicidae	3 species	1–4	1–3	1–4	1–4	1–4
Undetermined						
Formicidae		1–4	1–4	1–4	1–4	1–4
Paussidae	*Cochliopaussus klugi*					4
Scarabaeidae	*Metacatharsius, Aphodius, Schizonycha* spp	1–4	1–4	1–4	1–4	1–4

Numbers indicate the number of months after sowing. 1–4 means 1 to 4; i.e. throughout the period of observation; 1, 4 means 1 and 4, i.e. 1 month after sowing and 4 months after sowing.

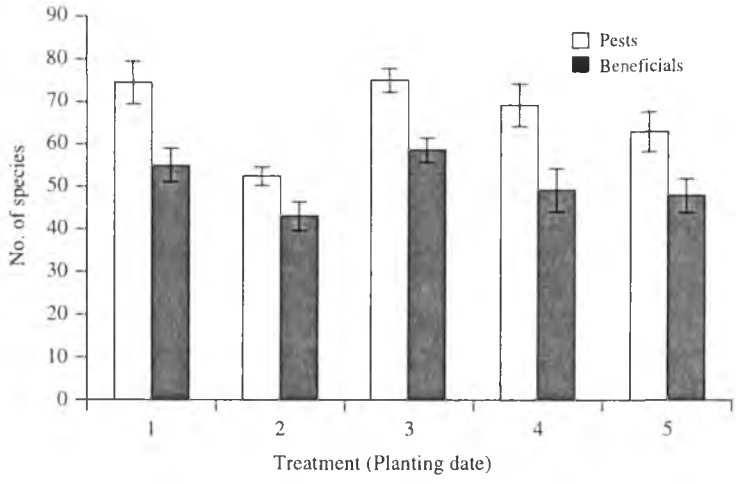

FIGURE 2. The total number of detrimental and beneficial species collected from each of treatment 1 = October 13, 2 = November 3, 3 = November 24, 4 = December 15, 5 = January 5.

Pest and Beneficial insects

For this analysis, only the dominant insect pests as well as those known to be pests on the groundnut (*Arachis hypogea* L.), a crop with similar growth and reproductive habits to the Bambara groundnut (Sesay *et al.*, 1999). The pest status of insects feeding on above-ground parts of the plant like the leaves, flowers and stems were was determined during visual observations, while the status of subterranean pests collected in the traps was determined from literature (Van Wyk and Cilliers, 1998; Wightman and Amin, 1988) (Table 1). While there were no significant differences ($P = 0.8980$) in the overall species diversity of insect pests (Fig. 2), there were differences in the resident pest species relative to the planting date. Observation of plants sown in October (treatment 1) indicated that insect damage was largely due to feeding on leaves and flowers by insect pests, e.g. Lepidopteran larvae like *Helicoverpa armigera* (Hubner), *Spodoptera* sp. grasshoppers (Acrididae) and Curculionidae (*Lixus* sp., *Blosyrinus* sp.). Within this treatment, $38.7 \pm 24.3\%$ of the plants had damaged leaves, where the leaves had holes or had only the main vein left at 4 weeks after sowing. In contrast, plants sown in January (treatment 5) had no leaf or flower damage at the same age. Instead, the dominant pests were the aphids, which formed colonies on the young leaves and stems. These pests cause leaf curling in young leaves and stems; $45 \pm 26.46\%$ of the plants in this treatment were infested with *Aphis craccivora* Koch.

In addition to the insect pests mentioned above, other potential pests on the crop were observed. These, however, occurred consistently throughout the experiment, e.g. subterranean pests including Tenebrionidae (*Gonocephalum* sp.), Termitidae (*Macrotermes* sp.), phytophagous pests including Curculionidae, (*Protostrophus* sp.,

Lixus sp., *Blosyrinus* sp.) and Gryllidae (crickets). While damage caused by these insects was noted, their effect on the final yield is yet to be determined.

The beneficial insect guild within the crop was also quite extensive. The most significant of these were the Coccinellidae, Carabidae, Reduviidae and Cicindellidae (*Dromica* sp.). Coccinellid species observed were *Hippodamia variegata* (Goeze); *Cheilomenes lunata* (Fabricius) and *Scymnus* spp. *H. variegata* and *C. lunata* are known to be aphidophagous and this correlated with observations that their peak densities occurred in the last treatment during the high aphid infestation. The voracious larval stage of these beetles was the most dominant during the period and this would have contributed towards aphid control in the final treatment, which was heavily infested. Carabidae and Cicindelidae are natural enemies of a variety of soft-bodied pest species, e.g. larvae of the Scarabaeidae and Lepidoptera, weevils, mealybugs and were observed throughout the period of observation. Pollinators, e.g. bees (Apidae) were also present on the crop, especially during the flowering phase of the crop. When coccinellids were excluded, there were no significant differences in beneficial insects' species densities between the treatments due to the planting date and age of the crop ($P = 0.5537$).

DISCUSSION

Results indicated that the pest species occurring during the first month after sowing was dependent on the oplanting date, e.g. only plants sown in December (treatment 5) has high aphid infestations at four weeks after sowing. These differences in pest species infestations at comparable ages may be attributed to the variation in occurrence of peak population densitites of the pest species. While some pest insects like lepidopteran and curculionid larvae emerge early in spring, groundnut aphids tend to become abundant after December (Annecke and Moran, 1982), causing leaf curling in young leaves and stunted growth (Borror *et al.*, 1989). None of the other treatments had any aphid infestations on the same date of observation nor at the same age of growth. The relationship between pest species and the planting date was also observed in the phytophagous beetle *Largria villosa.* where its densities were significantly lower in late-planted Bambara groundnut (Munthali *et al.*, 1996). Planting date of the Bambara groundnut may thus be planned to minimise the coincidence of the most deleterious insect pests with establishment of the plant, e.g. early planting could minimise damage due to aphid feeding.

It is possible that feeding by these insect pests would have adversely affected the final yield. Observations on the pigeon pea, *Cajanus cajan* (L.) Millsp. indicated that feeding by *H. armigera* and *Mylabris pustulata* Thunberg adversely affected the final yield of the crop (Marfo, 2000). Determination of the amount of damage caused by these insects and their effect on the final yield would ascertain if their activities warrant any control. The determination of the role of some of the insects observed would also serve to clearly define pests from beneficial insects, as well as those not likely to have direct effects on the plant. For example, *Cochliopaussus (Klugiopaussus) klugi* (Westwood) collected during this study, belongs to the family Paussidae, whose

members are know to have a close association with ants and are thus unlikely to have any significant interaction with the Bambara groundnut. Ants occurred in high densities in all treatments throughout the period of observation. Various ant species are known to tend honey-dew excreting insects, e.g. aphids, mealybugs and often do not directly cause serious damage on the crop itself. On groundnuts, ants are associated with *Hilda patruelis* Stal. (Tettigometridae) an insect pest which invariably leads to the death of the plants. Although their role on the Bambara groundnut was not ascertained in this study, they are however, known to also cause extensive damage on groundnuts (Annecke and Moran, 1982; Wightman and Amin, 1988; Van Wyk and Cilliers, 1998).

The difference in resident pest infestations due to date of planting indicates that the time of planting of the crop does have an effect on the pest fauna plaguing the crop. In Sierra Leone there is a wide range of time (months) within which farmers sow, with the intention to minimise pest infestations cited as one of the reasons for the variation (Sesay *et al.*, 1996). Further work needs to be carried out to ascertain the activitiy and population dynamics of these insects. This is especially important since Bambara groundnut is said to have minimal attack by insects while this study has illustrated that there are a variety of species, economically significant on crops like the groundunt (*A. hypogea*) and pigeon pea (*C. cajan*), that attack the crop. The activity of these species needs to be ascertained in order to determine if it warrants their control. Additionally, deleterious species like *A. craccivora* are known to also transmit viral diseases between leguminious plants (Wightman and Amin, 1988). Final yields are thus compromised, not only by feeding of these insects but also by the diseases they transmit. Investigation of the crop's yield from different planting dates relative to pest infestation woule help determine pest species and population densities that can be tolerated without significant losses in yield.

This preliminary study has shown that susceptibility of the Bambara groundnut to the various insect pests, e.g. aphids, bollworm and weevils seems to vary as the crop matures. The primary pest complex infesting the early planted treatment included the economically important *H. armigera* while the late planted treatment suffered heavy infestations by *A. craccivora*. Determination of the effect of these pests on the final yield would ascertain if these species warrant any control. Locally, the Bambara groundnut is cultivated as an income-generating crop so that in order to encourage its cultivation, economic input due to chemical use needs to be minimised. Minimising economic input for crop production, e.g. pesticide use, reduces the economic limitations which tend to hinder increased cultivation of the crop by subsistence farmers.

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Field trials of *Nosema locustae* for control of paddy grasshopper, *Hieroglyphus* spp. in Vidarbha region of India

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ABSTRACT: *Hieroglyphus banian* and *H. nigrorepletus* species of paddy grasshopper usually infest the paddy crop in Bhandara and adjoining districts of Vidarbha region in Maharashtra, India during June to October. Their population was estimated by monitoring two infested sites, Bamnee and Nerla from 1986 to 1989. The initial first instar infestation was approximately equal at Bamnee (9409/acre) and Nerla (9356/acre). The natural incidence of *Nosema locustae*, a microsporidian pathogen, was about 1–5% in *Hieroglyphus* population at both the sites. *N. locustae* spore suspension in water with 2% molasses was sprayed at one of the infested sites (Nerla) in 1986 and 1987 at the rate of 5×10^{10} spores/acre and in 1989, the dose was increased to 5×10^{11} spores/acre. The incidence of *N. locustae* infection increased to 29.5, 40.0 and 57.5% in 1986, 1987 and 1989, respectively, with maximum 82% hoppers and 73.3% adult population suppression in 1988. Increasing number of *N. locustae* spores were observed from field-collected infected grasshoppers each year. The results indicate that *N. locustae* as biocontrol agent can be used in long term suppression of grasshopper population due to its persistence in the field population through vertical transmission.

KEYWORDS: *Hieroglyphus* spp., *Nosema locustae*, Field trials, biocontrol, vertical transmission

INTRODUCTION

Nosema locustae (Canning, 1953), a microsporidian pathogen of locust and grasshopper has wide range of acridid hosts and extensive geographical distribution (Henry and Oma, 1981). It has been used as biocontrol agent against rangeland grasshoppers, *Melanoplus sanguinipes* and *M. infantilis* for the first time in USA (Henry, 1971). Its

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application later on extended to Argentina, Australia and China (Luna *et al.*, 1981; Moulden, 1981; Yu-hua, 1997; Lange, 1999; Lange *et al.*, 2000). In India, the paddy grasshoppers, *Hieroglyphus banian* and *H. nigrorepletus* in Bhandara and adjoining districts in Vidarbha region of India cause considerable damage to paddy crop, in pockets, and reduce paddy yield. A low rate of natural infection on *N. locustae* (1–5%) has been found in *Hieroglyphus* population in the field during 1984–85 (Raina *et al.*, 1987). Although *N. locustae* grows slowly and infected grasshoppers continue to cause damage to the crop, it persists in the population and is potentially useful for long-term control of grasshoppers (Henry and Oma, 1981; Lange, 1999). Therefore, attempts were made to assess the effectiveness of *N. locustae* in suppression of field population of paddy grasshopper in Vidarbha region of India. This paper describes the results obtained during field trials of *N. locustae* against *Hieroglyphus* spp. in the paddy crop.

MATERIALS AND METHODS

Nosema locustae production and formulation

N. locustae spores were isolated from *Hieroglyphus* spp. collected from the paddy field. The migratory locust, *Locusta migratoria migratorioides* was used as a host for mass production of *N. locustae* spores because its culture can be maintained throughout the year. The fourth instars of *L. migratoria* were inoculated per os via ingestion of 1 cm^2 pieces of *Sorghum* leaf dipped into suspension of 1.5×10^6 spores/ml in distilled water as described previously (Raina *et al.*, 1987). The wings and legs free infected adults of *L. migratoria* were triturated in distilled water. The homogenate was filtered through cheesecloth and left for about 24 h in the refrigerator. Subsequently, the layer of spores was separated and the spores were purified by repeated centrifugation method (Cantwell, 1970). The viability of spores was determined by counting shiny spores under phase contrast microscope and the spore virulence was tested by inoculating them to third instars of *L. migratoria* for infection. The spores were stored at $-10\,°C \pm 3\,°C$ in deep freezer.

Because of puddle cultivation of paddy, *N. locustae* on wheat bran could not be used and hence the spore suspension in water along with 2% molasses (used as sticker) was prepared for spraying on paddy crop.

Grasshopper population monitoring

The grasshoppers, *Hieroglyphus* spp. complete only one generation in a year from June to October and eggs laid by the female undergo diapause over the following winter and summer till the monsoon commences during next year. Hence the population monitoring and *N. locustae* applications were carried out from July to October in each year.

A survey of paddy growing areas in Bhandara district, about 95 km East of Nagpur was conducted in 1986 and two sites, Bamnee and Nerla were selected for the study. Both the sites had infestation of *Hieroglyphus* spp. One of the infested sites, Nerla was

selected for *N. locustae* field trials and the other site, Bamnee was left untreated and considered as control site.

Weekly survey of these sites was conducted from July to October during 1986, 87, 88 and 89 to estimate the grasshopper population. Sampling of grasshoppers was done using sweepnets, as well as visual counting at adult stage. Grasshopper population was estimated by counting grasshoppers collected at 10 randomly selected spots, each measuring about 500 sq. ft. At adult stage, visual count of grasshoppers was also added to the sweeping net collected ones. The grasshopper population per acre was then calculated as;

$$\text{Population/acre} = \frac{\text{Number of grasshoppers collected from 10 spots}}{\text{Area of 10 sampling spots } (10 \times 500 \text{ sq. ft.})} \times 43560 \text{ sq. ft. (area/acre).}$$

Field trials

In 1986, the spores were sprayed on the paddy crop on 2nd and 12th September when the grasshoppers were at late instar and adult stages. A total of 5×10^{10} spores/acre in suspension were applied in two sprays. In 1987, spraying was done with same rate once on 1st August when the hoppers were at early stage. To observe the persistence of infection in the field population, spraying of *N. locustae* was suspended during 1988. Again in 1989, the spraying continued with a higher dose. A total 10×10^{11} spores/acre were applied in two sprays on 31st July and 2nd August. The spores were sprayed using 20-liter capacity hand sprayer during evening. Weather condition was warm and moist with 20–60% daytime cloud cover, temperature 25–30 °C and relative humidity 70–90%.

Infection in field population

The infection rate in field population was estimated by collecting grasshoppers with sweeping net from each site before and after application of spores at regular intervals from 1986 to 89. Collected grasshoppers were homogenized individually and smears were observed under the microscope for infection. From the infected grasshoppers the spores were isolated and counted.

RESULTS

Population reduction

The data on population indicate slight fluctuations in the initial population at unsprayed control site (Bamnee) during July from 1986 to 89. At experimental site (Nerla) in 1986, the initial population was only 0.5% less than that of control site. The changes observed in population densities at unsprayed and *N. locustae* sprayed sites in each year were compared (Table 1).

In 1986, the maximum adult population was reduced by 23% at 4–5 weeks post spraying as compared to the unsprayed site. Consequently, at sprayed site in July 1987, the first instar population was reduced by 53.7% as compared to its initial population

TABLE 1. *Hieroglyphus* spp. population at *Nosema locustae* sprayed and unsprayed sites during 1986–89

Date of Survey	Population/acre unsprayed site (Bamnee)			Population/acre sprayed site (Nerla)		
	Hopper	Adult	Total	Hopper	Adult	Total
1986						
03 Jul	**9409**	0	**9409**	**9356**	0	**9356**
21 Jul	4414	0	4144	4292	0	4292
08 Aug	2614	0	2614	2638	0	2638
26 Aug	1882	78	1960	1870	0	1870
02 Sep	1315	514	1829	↓1498	↓244	↓1742
12 Sep	907	531	1438	↓ 928	↓397	↓1325
29 Sep	560	660	1220	580	535	1115
05 Oct	179	**701**	880	113	**540**	653
18 Oct	0	305	305	0	227	227
1987						
04 Jul	**8238**	0	**8238**	0	0	0
16 Jul	6500	0	6500	**5025**	0	**5025**
28 Jul	5234	0	5234	3850	0	3850
01 Aug	4696	0	4696	↓2984	0	↓2984
13 Aug	3029	0	3029	2489	0	2489
07 Sep	1093	405	2178	1113	125	1238
17 Sep	1051	377	1428	676	167	843
04 Oct	72	**475**	529	88	**197**	285
24 Oct	0	201	201	0	80	80
1988						
09 Jul	0	0	0	**1688**	0	**1688**
16 Jul	**9283**	0	**9283**	1198	0	1198
29 Jul	5314	0	5314	720	0	720
14 Aug	3108	0	3108	408	0	408
10 Sep	1789	219	2008	117	112	229
28 Sep	547	**516**	1063	36	**138**	174
23 Oct	0	286	286	0	102	102
1989						
11 Jul	**8150**	0	**8150**	**1923**	0	**1923**
31 Jul	NR	NR	NR	↓1049	0	↓ 1049
02 Aug	4486	0	4486	↓ 849	0	↓849
22 aug	2040	0	2040	523	0	523
01 Sep	1067	79	1146	346	39	385
21 Sep	456	190	646	56	**83**	139
07 Oct	**58**	**251**	309	3	81	84
27 Oct	0	63	63	0	19	19

↓–Application of *N. locustae* spores; NR–Not recorded

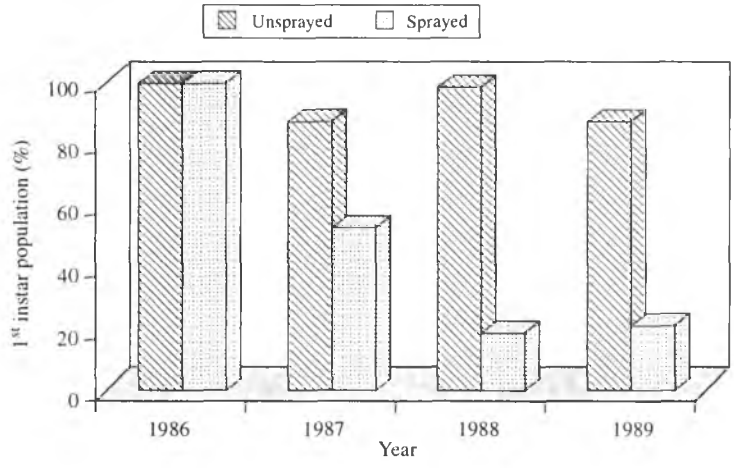

FIGURE 1. Effect of *N. locustae* spraying on 1st instar population of *Hieroglyphus* spp. during July from 1986 to 1989

in 1986, whereas at unsprayed site the reduction was only 13.4%. In 1987, the spraying was done at early hopper stages and resultant reduction in the adult population reached 58.5% at 9 weeks post-spraying. In 1988, at sprayed site, the reduction in first instar population in July was 82% as compared to its initial population in July 1986 (Fig. 1) and hence the spraying was suspended this year. In October 1988, the resultant maximum adult population was reduced by 73.3% compared to unsprayed site. In July 1989, the reduction in hopper population was 79.4% compared to its initial population in July 1986. This year the spraying was again continued with the increased dose of spores and the maximum adult population was reduced by 66.9% when compared with unsprayed site at 9 weeks post-spraying.

Infection

The grasshoppers collected after spraying from 1986 to 1989 showed clear evidence of infection. The data presented in Table 2 indicate that the average incidence of infection increased from 1.8% at pre-spraying to 29.5% post-spraying in 1986. In 1987, the infection rate increased to 40% and dropped to 26.5% in 1988 when spraying was suspended. With the resumption of spraying in 1989 the infection rate again increased to 57.5%. At control site the infection remained between 0.4 and 5.4% during 1986–89. The recovery of spores from infected grasshoppers varied according to the developing stages collected from the field (Table 2).

DISCUSSION

Hieroglyphus spp. infesting paddy field were found suitable for the assessment of effectiveness of *N. locustae* due to three reasons: (i) they complete only one generation

TABLE 2. *N. locustae* infection in the field population of *Hieroglyphus* spp. at sprayed and unsprayed sites during 1986–89

Year	Site	Number of grasshoppers			Number infected		*N. locustae* ($\times 10^4$)	
		Observed	infected	(%) Infection	Male	Female	Spores/ hopper	Spores/ adult
1986	Bamnee	245	1	0.41 ± 0.00	1	0	0	0
	Nerla*	275	5	1.80 ± 0.30	4	1	0	0
	Nerla↓	298	88	29.53 ± 3.57	58	30	0	0
1987	Bamnee	258	3	1.20 ± 0.20	3	0	0.022	0.70
	Nerla↓	472	189	40.04 ± 6.80	106	83	1.944	20.00
1988	Bamnee	270	13	4.82 ± 1.79	11	2	0.112	0.90
	Nerla	427	113	26.46 ± 1.75	73	40	8.290	51.43
1989	Bamnee	334	18	5.39 ± 0.46	16	2	0.220	0.67
	Nerla↓	527	303	57.49 ± 4.00	194	109	27.604	57.34

*Pre-spraying; ↓Post-spraying

in a year and all the developing stages could be monitored within 4–5 months, (ii) the population is confined to the field so that the effect of pathogen could be estimated accurately, (iii) the oviposition occurs in the muddy bunds surrounding the field which is advantageous to assess the infection due to transmission of pathogen in the progeny.

We suspect that the spores of *N. locustae* were effectively applied using spore suspension in water with 2% molasses for gluing the spores on the leaves. The viability did not decrease below 90% even after 60 h post-spraying during monsoon (July–September) when temperature ranged between 25 and 30 °C with high humidity (70–95%) (Rai *et al.*, 2000).

The application of higher doses of *N. locustae* (5.0×10^{10} and 5×10^{11} spores/acre) ensured retention of maximum number of spores on the leaf surface for ingestion by the grasshoppers. Reuter *et al.* (1990) reported that the standard application rate of *N. locustae* (1.0×10^9 spores/acre) was too low to induce immediate suppression of rangeland grasshopper population when they evaluated field population after receiving either the standard rate (1.0×10^9 spores/acre) or higher rate (1.0×10^{11} spores/acre).

The present study demonstrated that application of *N. locustae* spores was effective in reducing the grasshopper population in the field. A slight reduction in total population with less infection rate in 1986 might be due to application of *N. locustae* at the late hopper and adult stages. Usually spores are detected about 3 weeks after application in the infected grasshopper (Henry and Oma, 1974). Most protocols recommended microscopic examination at 28 days following application (Henry, 1978). The reduction in adult population and increase in infection rate from 1987 to 1989 might be achieved by application of pathogen at early instars. In the laboratory *Melanoplus* spp. and *L. migratoria* infected as third instar or younger develop slowly and usually die during fourth and fifth stage (Henry, 1971; Raina *et al.*, 1987).

The low density of 1st instar following application of *N. locustae* in 1987, 1988 and 1989 might be attributed to, less egg production by infected female survivors. Henry (1969, 1971) detected little ovarial or egg debris in infected females and suggested that infected females fail to develop reproductively. Even and Mukerji (1980) reported substantially lower rates of egg laying after application of *N. locustae* in the field. Lockwood and DeBrey (1990) also observed some evidence of lower egg production in higher population of grasshoppers treated with *N. locustae*. In the present study, persistence of infection in the grasshopper population in 1988 without application of *N. locustae* indicates the possibility of transmission of *N. locustae* in field population via eggs. The new population emerged directly from the eggs laid by the adults of previous generation had shown the infection. Even and Mukerji (1980) were unable to find spores in eggs collected from *N. locustae* treated plots but they did observe infection among the instars raised from field collected eggs. Henry and Onsagar (1982) also reported infection in grasshopper population during the year after treatment. Although the mechanisms and the rate of *N. locustae* transmission in the field have not been studied adequately, a mechanism of transovarial transmission of *N. locustae* in *L. migratoria migratorioides* has been studied in the laboratory by Raina *et al.* (1995). They reported the incidence of infection ranging from 72–92% among the progeny up to F_{14} generations.

The epizootic of *N. locustae* infection in grasshopper population in the field even after years of application has been reported by Yu-hua (1997) in China and Lange (1999) in Argentina. The transovarial transmission may be one of the means by which *N. locustae* persists in the population for longer period. Therefore, its use as biocontrol agent must be considered as part of long-term strategy for suppression of grasshopper population (Vaughn *et al.*, 1997).

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Aggregation activity induced by the excreta extracts in *Cimex hemipterus* (Hemiptera: Cimicidae)

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ABSTRACT: Attempts were made to ascertain the presence of semiochemicals responsible for aggregation in excreta, body emanations, macerated body extract, and whole body extract of bedbug, *Cimex hemipterus* using hexane (non-polar), dichloromethane (semi-polar), methanol (polar) and water (highly polar) as solvents for extraction with the objective of finding suitable solvent for extraction. Out of the four solvents, water and methanol extracts of filter papers containing excreta of bedbugs gave very high value of attraction index for aggregation in case of male, female and fifth nymphal instar indicating the presence of semiochemicals responsible for aggregation, while extracts of body volatiles, whole body and macerated body did not elicit positive response. EAG studies of the methanol extracts of the filter paper containing excreta also indicated strong positive response in case of male and female bedbugs.

KEYWORDS: Excreta extracts, *Cimex hemipterus*, aggregation pheromone, bedbug

INTRODUCTION

The nocturnal biting of bedbugs is debilitating to humans whose sleep is disturbed every night. In addition to causing annoyance, bedbugs are suspected to be vectors of many diseases. Primarily, they are of medical importance because of their irritating bite and subsequently secondary infections that may occur due to irritation of the skin. The morphological and physiological characteristics, adaptability from one host to another and piercing and sucking mouthparts of the bedbugs make this insect a potential vector of blood infecting diseases. Experimentally bedbugs have been infected with many human pathogens such as Hepatitis-B, HIV and *Trypanosoma cruzi*, which cause Chagas's disease (Wills *et al.*, 1977; Jupp *et al.*, 1978; Ogston and London, 1980). The status of bedbugs as vectors of disease was summarised by Usinger (1966). Although the role in the transmission of various diseases is not yet

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clearly understood, the topic is still of public health importance (Harwood and James, 1979). Nevertheless, their possible control by manipulation of semiochemicals has received surprisingly little attention.

Marx (1955) reported that the gregarious habit of *Cimex lectularis* is promoted by the presence of a specific nest odour, which could be provided also by crushed bedbugs in contradiction to the previous investigators (Kemper, 1936). Collins (1968) reported that the stink emitted by *C. lectularis* was very offensive and was due to the carbonyl compounds produced by it. Later Levinson and Barllan (1971); Levinson *et al.* (1974) attempted to clarify the somewhat confusing situation by studying the olfactory behaviour of bedbugs towards their body scent in *C. lectularis*. Unfortunately, no effort was made further to study the semiochemicals of bedbugs. Therefore the present study is undertaken to identify the source of aggregation factor(s) responsible for aggregation in case of different stages of bedbug, *Cimex hemipterus* by (i) extraction from filter paper in which bedbugs were maintained for 10 days using different solvents with the point of view of identifying suitable solvent for isolation of aggregation factor; (ii) collection of volatiles/emanations from bedbugs and (iii) whole body extraction.

MATERIALS AND METHODS

Insects

Cimex hemipterus adults and nymphs were obtained from insectary colonies maintained at room temperature (27 ± 1 °C) and relative humidity (70 ± 5%) in diffused daylight conditions. They were cultured by feeding with rabbit blood through a nylon cloth according to the method described by Damodar *et al.* (1962).

Collection of the excreta

The faeces of adult males, females and nymphs were extracted from the filter paper taken from the chambers in which insects were maintained for 10 days continuously. Four different solvents were used for the extraction of the possible attractive substances: Hexane (non-polar). Dichloromethane (semi-polar), methanol (polar) and water (highly polar). All the solvents were of HPLC grade. Extractions were made using 15 ml of solvent, and from filter paper used for 500 bed bugs in a single chamber.

Collection of volatile substances

Volatile substances secreted by the insect or released from the excreta of bed bugs were collected by a modification of the technique of Yamamoto (1963) using XAD-2 solid adsorbent. Air was passed at the rate of ca 1 litre/minute from an air compressor, into the glass chamber containing 500 bed bugs, either male or female maintained at 27 ± 1 °C. The outlet of the insect chamber was connected to the trap containing XAD-2 adsorbent. Air was passed continuously for 10 days. The insects were fed on rabbits once in three days, the dead bed bugs were replaced everyday to keep the

number of insects same [The adsorbent XAD-2 used for collection of volatiles was cleaned with the methanol by soxhlet extraction; and typically, a large active carbon filter was employed to ensure that any volatiles in the air supply were removed prior to its passage into the insect chamber]. The adsorbed volatiles from the XAD-2 were extracted with 3 × 5 ml of methanol (HPLC grade) and used for bioassays.

In another experiment, activated charcoal was used for collecting the volatiles instead of XAD-2. Also the volatiles were collected using a trap containing methanol, kept at −10 °C instead of solid adsorbents (XAD-2 & Activated charcoal).

Whole body extraction

Bed bugs (500 nos., male, female and nymph each separately) were taken in a test tube, previously well cleaned with methanol; 15 ml of methanol (HPLC grade) was added to it, the solvent was decanted immediately into another tube and used for bioassays.

Electroantennography

The antenna of unfed male/female bedbug was carefully removed with the help of Iris scissor and fine forceps under a microscope in Beadle-Ephrussi-Ringer solution (0.35 g KCl, 7.54 g NaCl, 0.21 g, $CaCl_2$ in 1 litre distilled water). Its base was carefully slided inside the tip of micropipette filled with Ringer solution of the recording (different) electrode and the tip of antenna was brought in contact of micropipette with Ringer solution of reference (indifferent) electrode with the help of a manipulator of EAG system (Syntech, The Netherlands) under stereomicroscope. Silver wire (0.25 mm diameter) inserted at the ends of the capillary electrodes were used to make the connections between the electrodes and coaxial cables to the amplifier (probe). The signals were recorded on interfaced computer.

For EAG recordings, test solutions (10 μl methanol) were applied to a zigzag folded filter paper (1 × 5 cm) which is partly inserted in the wide opening of the Pasteur pipette and solvent was allowed to evaporate and then paper was pushed completely inside the pipette. The Pasteur pipette was attached to a solenoid-controlled air flow and a 0.3-s puff was directed over the antennal preparation through a small hole in the glass tube (1 cm i.d.) delivering a continuous flow of purified moistened air (114 ml/min) over the antennal preparation.

Behavioural test analysis

The aggregation activity of bed bugs was determined by a modified technique based on the choice chamber method essentially described by Shri Prakash *et al.* (1990) used for screening repellents for cockroaches with some modifications. The apparatus was designed in our laboratory to test the biological activity of the various samples. It consists of five glass chambers. The central chamber was the largest, 9 cm height and 3.5 cm diameter to which four small jars were connected on the four sides separately through a tube of 3 cm length and 1 cm diameter. The tops of the all chambers were closed with muslin cloth. In the four arm apparatus, two jars contained blank filter

papers, one jar contained control filter paper and one jar contained solvent extract treated filter paper.

Conditioned filter papers for aggregation activity studies were prepared from each solvent extract. For this 500 μl of the extract were used for impregnating the conditioned paper and it was left in the room temperature until the solvent had completely evaporated. Control papers were prepared by impregnating the filter paper with 500 μl of pure solvent and left until the solvent had completely evaporated. They were used for bioassays immediately.

For the bioassay, 30 bed bugs of same age group (male or female or nymph) were placed in the central chamber after keeping the control and treated papers in the side arms. The number of bed bugs entering the different jars was recorded after an interval of 30, 60 and 120 minutes. There were four replicates in each assay.

The basis for calculating aggregation activity was the number of bedbugs in both control and treated arena. The aggregation activity was expressed as attraction index (AI) and calculated as follows: $AI = T - C/T + C$, where T denotes the mean number of bedbugs in the treated arena and C denotes the mean number of bedbugs in the control arena (Kramer and Mulla, 1979).

All the index values fall within the range of $+1$ to -1. A positive value indicates that more aggregation occur in the treated than in the control, thus signaling the treated extract to be attractive. Conversely, more aggregation in the control than in the treated would result in the negative AI value, indicating the extract to be non-attractive. However, the attraction is considered to be practically significant if $AI > +0.25$.

RESULTS

These experiments were aimed, first, to find the most efficient solvent for extracting the semiochemicals, which induce bedbugs to aggregate, and second, to confine the production site(s) or mode of release of the aggregation pheromone and then to analyse it.

Efficiency of solvents in extracting the attractive substances from excreta

Table 1 shows the total number of female, male and nymph in control and treated arena of choice chamber and respective value of attraction index of excreta extracted from filter paper with various solvents. The dichloromethane and hexane extracts elicited negative response indicating absence of any possible aggregation factor in these extracts irrespective of the stage/sex of bedbug. However in case of methanol and water extracts, the number of females, males and nymphs was higher in treated arena than in control arena leading to higher and positive value of attraction index, varying from $+0.90$ to $+0.94$ with methanol extract and $+0.95$ to $+0.98$ with water extract, suggesting presence of some chemical aggregation factor(s) in methanol and water extracts of bedbug excreta.

Aggregation efficiency of methanol extracts of volatiles emanated and whole body

The values of attraction index of methanol extract of whole body wash from female, male and nymphs as well as volatiles collected from the males and females are given

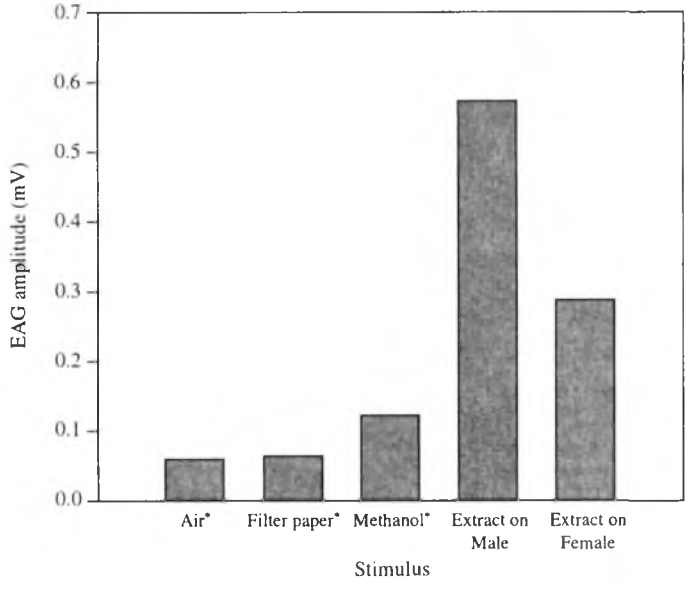

FIGURE 1. Electroantennogram (EAG) responses of male and female antenna of bedbug. *Cimex hemipterus* to methanol extracts of its excreta (* on male antennae).

TABLE 1. Attraction indices of the excreta of bedbug *Cimex hemipterus* extracted with different solvents

Stage of the bedbug	Solvent	Number of Bedbugs in		Attraction index
		Control arena	Treated arena	
Female	Dichloromethane	43.6 ± 1.4	24.0 ± 1.8	−0.29
	Hexane	68.2 ± 2.2	4.3 ± 0.38	−0.88
	Methanol	82.2 ± 4.4	3.4 ± 1.6	+0.92
	Water	86.7 ± 8.9	1.8 ± 0.2	+0.96
Male	Dichloromethane	43.2 ± 0.83	41.4 ± 3.6	−0.02
	Hexane	29.0 ± 4.9	25.1 ± 0.9	−0.07
	Methanol	2.8 ± 0.1	84.4 ± 1.6	+0.94
	Water	1.1 ± 0.08	87.0 ± 2.5	+0.98
Nymphs	Dichloromethane	26.0 ± 4.9	12.8 ± 1.6	−0.34
	Hexane	20.1 ± 2.5	15.0 ± 3.3	−0.15
	Methanol	3.4 ± 0.2	82.2 ± 1.7	+0.92
	Water	2.2 ± 0.1	85.2 ± 1.8	+0.95

in Table 2. In all cases, irrespective of the stage or sex of the bedbug, the values of attraction index were observed to be negative. These negative values of attraction index suggest that aggregating factor may not be present.

TABLE 2. Attraction indices of methanol extract of female, male and nymphal stages of bedbug *Cimex hemipterus* obtained from whole body extraction, maceration and volatiles

Nature of the extract	Stage/sex	Attraction index
Whole body extract	Female	−0.33
	Male	−0.10
	Nymph	−0.09
Macerated body extract	Female	−0.36
	Male	−0.55
	Nymph	−0.16
Volatiles collection		
(i) XAD-2 extract	Female	−0.64
	Male	−0.94
(ii) Activated charcoal extract	Female	−0.42
	Male	−0.64
(iii) Solvent trap	Female	−0.12
	Male	−0.22

EAG studies

Antennae from unfed male/female bedbugs responded strongly to the methanol extract of filter paper containing excreta (Fig. 1). However, the response is more prominent in male than female. The antennae did not show any response to hexane or dichloromethane extracts.

DISCUSSION

Results of behavioural tests showed that water and methanol were efficient in extracting active substances from excreta which induce aggregation. Hexane or dichloromethane were not efficient and were therefore discarded from further experiments. The antennal responses in EAG recordings are also specific to methanol extracts; the male antenna evoked higher EAG response than female antenna.

Hexane is a non-polar solvent and normally extracts non-polar hydrocarbons; dichloromethane is a medium polar solvent and extracts polar compounds along with some non-polar compounds including hydrocarbons; methanol and water are polar solvent and extract polar substances. Our results showed that the two solvents that extract polar molecules are the most efficient for extracting the attractive substances. Since methanol is gas chromatography amenable and the attraction index is not much different from that of water extract, all further experiments were carried out with this solvent.

We have attempted to find the mode and the site of production of the attractive substances by extracting the whole body and volatile emanations with methanol. However, the failure of these suggests that the activity of aggregation factors may be present in the excreta extract. Levinson and Barllan (1971) reported that bedbugs, *C. lectularis* of both sexes assembled at similar or equal rates under papers, which had been scented by the odour of 25 female or male bedbugs but could not find out the origin of the odour. Schofield and Patterson (1977) reported the presence of assembly pheromone in faeces of unfed nymphs of *Triatoma infestans* and *Rhodnius prolixus*. Lorenzo and Lazzari (1996) observed assembly of *T. infestans* bugs around their faeces and considered the role of excrement as a chemical signal or assembly. Also in case of some species of cockroach, presence of aggregation pheromones in faeces has been reported by various workers (Ishii, 1970; Sommer, 1974; Metzger and Trier, 1975; Miller *et al.*, 2000). The pads present in the rectum of *Blatella germanica*, which were comprised of 'glandular cells', were assigned the role of production of juvenile assembly pheromone which comes out along with excreta (Ishii and Kuwahara, 1967, 1968). The aggregation factors present in methanol and water extract of faeces of nymphs, male and female of *C. hemipterus* may be the products formed as a result of breakdown of blood meal or may be added in the posterior gut or hind gut before defecation by so far unknown mechanism.

The negative response of whole body wash is similar to the results obtained by Levinson *et al.* (1974) in case of *C. lectularis*. They observed alarming action induced by the scent gland secretions. We conclude that the methanol extract of the excreta shows highly positive aggregation behaviour in bedbug. *C. hemipterus* which may be further analysed to identify the chemical component/semiochemical responsible.

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A key and a checklist of the genera of short-horned grasshoppers (Orthoptera: Acridoidea) of Kerala

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ABSTRACT: A dichotomous key for the easy identification of the genera of short horned grasshoppers of Kerala is provided. A checklist of the genera of Acridoidea is also given.

KEYWORDS: Acridoidea, generic key, checklist-Kerala

INTRODUCTION

Short-horned grasshoppers form a major pest of crops. Taxonomic studies of short horned grasshoppers hence become relevant in the control of these pests.

Ander (1939); Chopard (1949); Bay Bienko and Mistshenko (1951) and Uvarov (1966) included the short-horned grasshoppers under the superfamily Acridoidea of the suborder Caelifera. Dirsh (1961) considered Acridoidea as Suborder.

In the Fauna of British India Kirby (1914) has reported 91 genera under Acridoidea. In the present paper twenty-eight genera are reported from Kerala. A dichotomous key for the easy identification of these genera is provided in this paper along with a checklist of the genera of Acridoidea of Kerala.

Key to the genera of Acridoidea of Kerala

1. Face mostly flattened, cubital vein of tegmina (Fig. 1) and medial vein of hind wing unbranched. (Fig. 2). Antenna shorter than front femur; basal segment of hind tarsus with serrated margins (Fig. 5) or with teeth or atleast with a basal external tubercle ..... **Eumasticidae** **2**

   Face not flattened, cubital vein of tegmina (Fig. 3) and medial vein of hind wing usually branched, (Fig. 4) antenna longer than front femur, basal segment of hind tarsus never serrated, never with tooth or tubercle **3**

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2(1) Pronotum tectiform (= roof-like) and foliaceous; hind femora dilated and compressed ..... ***Phyllochoreia*** **Westwood**

Pronotum not as above and hind femora not dilated ..... ***Erianthus*** **Stal**

3(1) Fastigial furrow (Fig. 6) present; apical areolae (Fig. 6) generally present; lower basal lobe of hind femur longer than upper lobe ..... **Pyrgomorphidae** **4**

Fastigial furrow absent; apical aerolae absent, lower basal lobe of hind femur shorter than or as long as upper lobe ..... **Acrididae** **9**

4(3) Tegmina and wings absent **5**

Tegmina and wings present **6**

5(4) Fastigium horizontally elongated; antenna filiform; Pronotum without carina ..... ***Orthacris*** **Bolivar**

Fastigium longitudinally elongated; antenna basally ensiform; Pronotum with weak carina ..... ***Neorthacris*** **Kevan and Singh**

6(4) Anterior margin of pronotum forming wide collar, covering posterior and lower part of mouth; tympanum absent ..... ***Chrotogonus*** **Serville**

Anterior margin of pronotum not covering posterior and lower part of mouth; tympanum present **7**

7(6) Body slender; antennal bases located infront of lateral ocelli .....

..... ***Atractomorpha*** **Saussure**

Body robust; antennal bases located between or behind lateral ocelli .. **8**

8(7) Posterior lobe of pronotum convex raised above level of anterior lobe; with strong rugae ..... ***Aularches*** **Stal**

Posterior lobe of pronotum level, not raised; without rugae .....

..... ***Poekilocerus*** **Seville**

9(3) Prosternal process present; antenna filiform **17**

Prosternal process absent; if present antenna ensiform, body elongate ..... **Acridinae** **10**

10(9) Antenna ensiform **11**

Antenna filiform **14**

11(10) Prosternal process present ..... ***Gelastorrhinus*** **Brunner**

Prosternal process absent **12**

12(11) Head conically ascending (Fig. 7) apical areolae present; posterior angle of pronotum acutely angulated (Fig. 8) ..... ***Acrida*** **Stal**

Head not conically ascending; apical areolae absent; posterior angle of pronotum not acutely angulated **13**

13(12) Wings and tegmina well developed ..... ***Phlaeoba*** **Stal**

Wings and tegmina not fully developed ..... ***Phlaeobida*** **Bolivar**

14(10) Median carina of pronotum well raised; wings basally yellow and with well marked longitudinal band of fascia in the middle ..... ***Gastrimargus*** **Saussure**

Median carina of pronotum not raised; wings without longitudinal band of black fascia in the middle **15**

15(14) Pronotum above with light yellow 'x' shaped marking (Fig. 9) ..... ***Oedaleus*** **Fieber**

Pronotum without 'x' shaped marking **16**

16(15) Median carina in prozona forming two tooth-like projections ..... ***Trilophidia*** **Stal**

Median carina of pronotum in prozona not forming tooth-like projections ..... ***Dittopternis*** **Saussure**

17(9) Lower external lobe of hind knee with spine like apex .... (Fig. 10) .... **Oxyinae** **18**

Lower external lobe of hind knee with rounded angular or subacute apex, never spine like **19**

18(17) Wings and tegmina well developed; male terminalia without furcula ... . ***Oxya*** **Serville**

Wings and tegmina not well developed; male terminalia with node like furcula ..... (Fig. 11) ..... ***Cercina*** **Stal**

19(17) Radial area of tegmina with series of regular parallel stridulatory veinlets ..... **Hemiacridinae** **20**

Radial area of tegmina without a series of regular parallel stridulatory veinlets **21**

20(19) Dorsum of pronotum flat with well developed median and lateral carinae; prosternal process spatulate; male cercus simple, conical; tegmina with nervures ..... ***Spathosternum*** **Karsch**

Dorsum of pronoum rounded with weak median carina; lateral carinae lacking; prosternal process conical; male cercus bifurcate; tegmina without nervures ..... ***Hieroglyphus*** **Krauss**

21(19) Mesosternal interspace closed (Fig. 12) ..... **Tropidopolinae**
..... ***Oxyrrhepes* Stal**

Mesosternal interspace open (Fig. 13)..... **22**

22(21) Mesosternal lobes rectangular (Fig. 13); head and pronotum with light coloured median stripe..... **Cyrtacanthacridinae**..... **23**

Mesosternal lobes rounded (Fig. 14) or obtuse angular not rectangular; head and pronotum without light coloured median stripe **24**

23(22) Prosternal process straight, conical or subconical ..... ***Patanga* Uvarov**

Prosternal process strongly curved backwards, touching mesosternum, inflated in the middle..... ***Cyrtacanthacris* Walker**

24(22) Dorsum of pronotum flat or weakly tectiform with median and lateral carinae ..... **Eyprepocnemidinae**..... **25**

Dorsum of pronotum variable shape, lateral carinae absent.....
Catantopinae..... **26**

25(24) Hind tibia with dense spines; interspace between outer row of spines equal to their basal width; prosternal process spatulate or trilobate; hind margin of subgenital plate of female entire and single lobed.....
..... ***Tylotropidius* Stal**

Hind tibia with sparse spines; interspace between outer row of spines twice the width of base of spines; subgenital plate of female trilobate...
..... ***Eyprepocnemis* Fieber**

26(24) Head short and very broad; face broader than long..... ***Euthymia* Stal**

Head not remarkably long..... **27**

27(26) Wings and tegmina well developed ..... ***Catantops* Schaum**

Wings not well developed, tegmina rudimentary..... ***Mesambria* Stal**

Checklist of the genera of the suborder Acridoidea of Kerala

Family: Acrididae

(i) Subfamily: Acridinae

1.	*Acrida* Stal 1873	Asia, Australia, Africa
2.	*Dittopternis* Saussure 1884	India, Sri Lanka, Australia, S. Africa
3.	*Gastrimargus* Saussure 1884	Asia, Australia, Africa
4.	*Gelastorrhinus* Brunner 1903	India, Africa, Madagascar
5.	*Oedaleus* Fieber 1835	Asia, Australia, Africa
6.	*Phlaeoba* Stal 1860	Oriental region
7.	*Phlaeobida* Bolivar 1902	India

8.	*Trilophidia* Stal 1872	Oriental region
(ii) Subfamily: Catantopinae		
9.	*Catantops* Schaum 1853	Africa, Oriental region, Australia
10.	*Euthymia* Stal 1875	India, Sri Lanka, Madagascar, Borneo
11.	*Mesambria* Stal 1878	India, Sri Lanka, Madagascar, Celebes
(iii) Subfamily: Cyrtacanthacridinae		
12.	*Cyrtacanthacirs* Walker 1870 = *Acrydium* Oliver 1791 = *Acridium* Uvarov 1831	Oriental region
13.	*Patanga* Uvarov 1923	India
(iv) Subfamily: Eyprepocnemidinae		
14.	*Eyprepocnemis* Fieber 1853	S. Europe, Africa, W. Asia, Oriental region
15.	*Tylotropidius* Stal 1873	Africa, Sri Lanka, Burma, India
(v) Subfamily: Hemiacridinae		
16.	*Heiroglyphus* Krauss 1877	China, India, Burma, Senegal
17.	*Spathosternum* Karsch 1877	India, Siam, W. Africa
(vi) Subfamily: Oxyinae		
18.	*Cercina* Stal 1878	Sri Lanka, India
19.	*Oxya* Serville 1831	Africa, Asia, Australia
(vii) Subfamily: Tropidopolinae		
20.	*Oxyrrhepes* Stal 1873	Oriental region, Africa
Family: Eumasticidae		
21.	*Erianthus* Stal 1875	Indo Malayan region
22.	*Phyllochoreia* Westwood 1839	India, Sri Lanka, Borneo
Family: Pyrgomorphidae		
23.	*Atractomorpha* Saussure 1861	Ethiopian, Australian and Oriental region
24.	*Aularches* Stal 1873	India
25.	*Chrotogonus* Serville 1839	Africa, Australia, Asia
26.	*Neorthacris* Kevan and Singh	India
27.	*Orthacris* Bolivar 1884	India, Sri Lanka
28.	*Poekilocerus* Serville 1831 = *Poecilocerus* Stal 1873	India, W. Asia, North East Africa

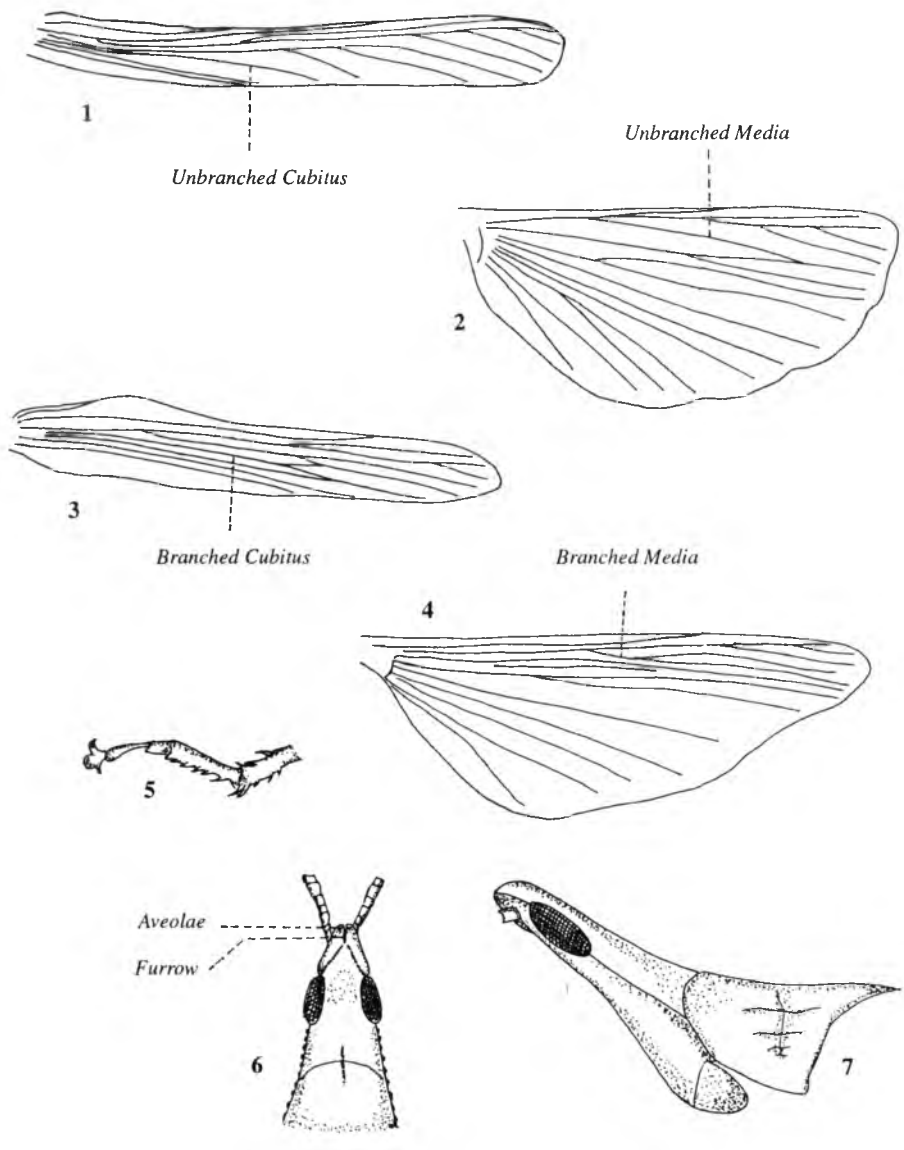

FIGURE1–7: 1. Tegmina with unbranched cubitus; 2. Hind wing with unbranched media; 3. Tegmina with branched cabitus; 4. Hind wing with branched media; 5. Basal segment of hind tarsus with serrated margins; 6. Dorsal view of head showing fastigial furrow and areolae; 7.Lateral view of conically ascending head.

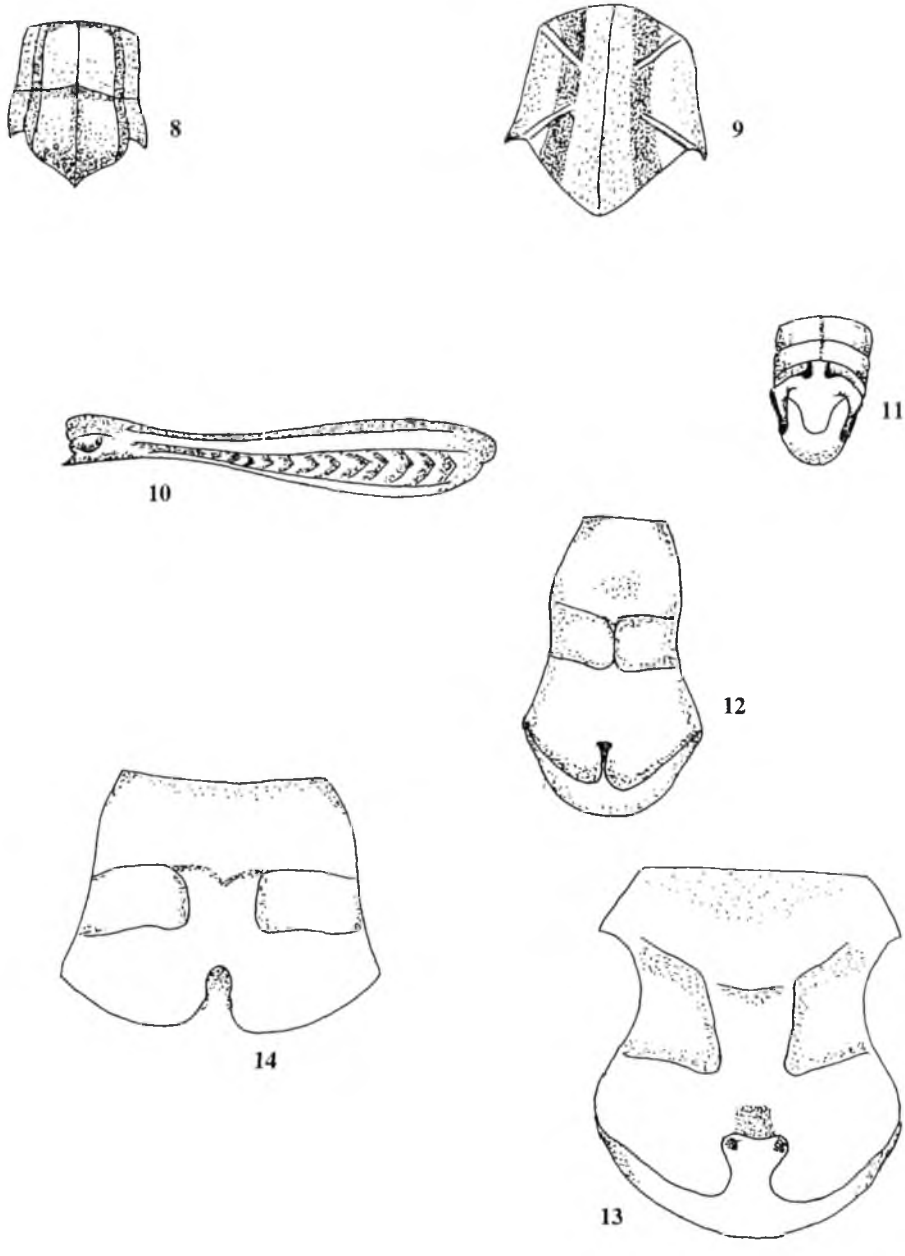

FIGURE8–14: 8. Acutely angulated pronotum; 9. Pronotum with x shaped marking; 10. Lower external lobe of hind knee with spine like apex; 11. Male terminalia with node like furcula; 12. Closed mesosternal interspace; 13. Rounded mesosternal lobes with open interspace; 14. Rectangular mesosternal lobes.

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Effect of microbial infection on the posterior silk gland in the tropical tasar silkworm, *Antheraea mylitta* (Drury) (Lepidoptera: Saturniidae)

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ABSTRACT: The EM studies reveal the presence of bacteria, virions of nuclear polyhedrosis virus (NPV) and spores of *Nosema* sp. in the cells of posterior silk gland (PSG). The bacteria are gram-positive, rod-like bacilli with extensively long flagella. They often form a sort of chain in the cells. The virions or capsids of NPV appear as the double rod-shaped structures. The spores of the monosporoblastic microsporidian, *Nosema* sp. are oval in shape. The cells of the PSG infected with the bacilli show large number of lysosomes and phagocytosomes in close vicinity of a chain of bacilli suggesting the well-defined cellular defense mechanism. Infection of bacteriosis and virosis causes significant reduction in total protein concentration in the PSG.

KEYWORDS: *Antheraea mylitta*, silk gland, microbial infection

Microbial diseases of the tropical tasar silkworm, *Antheraea mylitta* causing heavy loss of the crop in India is found every year particularly during the adverse environmental conditions (Sen *et al.*, 1969; Shukla *et al.*, 1994; Hatakeyama *et al.*, 2000). Information on the cytomorphology, taxonomy and pathogenic effects of microbial pathogens in *A. mylitta* is, perhaps, very meager (Jolly and Sen, 1972); (Bansal *et al.*, 1997).

The present study was, therefore, undertaken to explore ultrastructure of the microbial pathogens to elucidate their cytomorphological structure and their pathogenic effects on the cell organelles and total protein concentration in the posterior silk gland (PSG) in *A. mylitta*.

The healthy and infected fifth instar larvae of trivoltine tropical tasar silkworm, *A. mylitta* (Daba race) were brought to the laboratory from the field. The infected larvae suffering from bacteriosis, virosis and microsporidiosis were recognized from their external characteristics (Jolly *et al.*, 1979). The silk glands were dissected out gently from 10 to 12 healthy and equal number of the larvae suffering from each

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disease. After removing the fat body and trachea, the posterior silk glands (PSG) were separated and divided into two equal groups and used for transmission microscopy and estimation of total protein concentration respectively.

The PSG dissected from various groups of larvae were fixed in Karnvosky's fixative for a period of 18 hr. The material was thoroughly washed and post-fixed in 1% osmium tetraoxide dissolved in Karnvosky's fixative. The material was stained *en block* with 0.5% Uranyl acetate during dehydration with graded alcohols and embedded in araldite or styrenemetacrylate. The ultra-thin sections were cut on the Rieckert ultra-cut-s' LKB ultramicrotome and scanned under EM 'JEOL, 1200 EX-II' (Japan) at desirable magnification at the Regional Sophisticated Instrumentation Centre (RSIC), Nagpur University, Nagpur (India).

The PSG dissected from various groups were transferred into 2 ml ice-cold phosphate buffer saline (pH 7.03) separately after weighing to (0.001 mg) accuracy. The material was homogenized for 3 min at 0 °C with the help of glass-homogeniser. The homogenized samples were centrifuged at 7000 rpm for 10 min at room temperature and total protein concentration was estimated by the method of Lowry *et al.* (1951).

The EM studies on PSG of the diseased larvae showed the presence of large number of darkly stained, gram-positive, rod-like cylindrical bodies measuring about 6.26 μm in length and 2.33 μm in diameter and often with one broad end (2.99 μm). Bacilli are linked to each other forming a sort of chain (Fig.1). The bacilli are provided with extensively elongated flagella (Fig. 2).

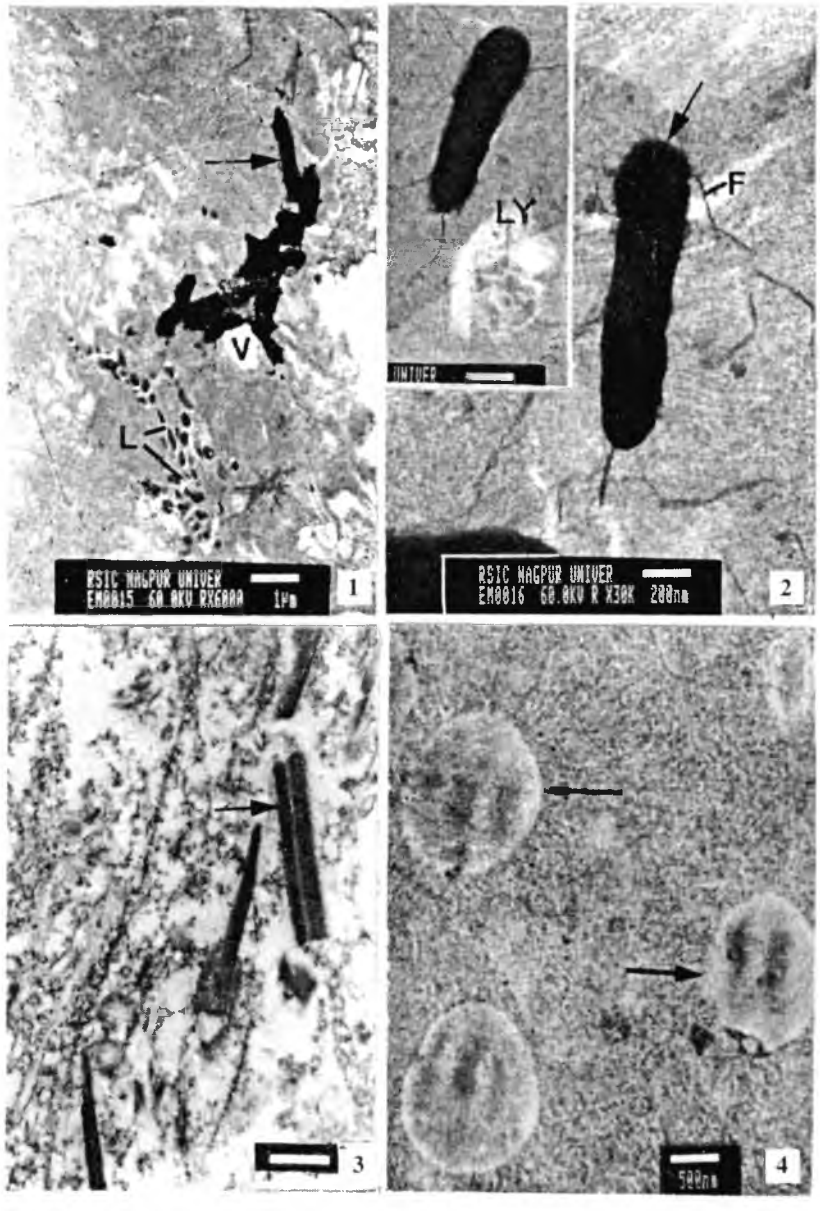
The infected cells of PSG contain large number of vacuoles, scanty cytoplasm and disintegrated cytoplasmic organelles. Simultaneously, the lysosomes appear in close vicinity of the bacilli. Some phagocytotic bodies and vacuoles are also well evident adjacent to the bacterial clusters.

EM studies on PSG in the larvae suffering from virosis showed presence of well-stained virions or capsids of NPV as the double rod-shaped structures measuring about 5.642 μm in length and 1.285 μm in width. The microtubules, Golgi bodies, rough endoplasmic reticulum (RER) and other cytoplasmic organelles as well as cytoplasm of the infected cells of PSG become completely regressed. Empty membranous vesicles can be seen abundantly.

EM studies on PSG in the larvae suffering from microsporidiosis revealed the presence of large number of oval-shaped spores of *Nosema* sp. measuring about 1.83 μm in length and 1.53 μm in width at the middle. Due to infection, the cytoplasmic organelles are greatly disintegrated and severe sub-cellular destruction of the silk gland cells by the pathogens is well evident (Fig. 4).

Significant reduction was observed in the total protein concentration in PSG of the larvae infected with bacteriosis and virosis in comparison to that in the control (healthy) larvae. Moreover, no significant change was found in the larvae suffered with microsporidiosis (Table 1).

In India, the tasar silkworm, *A. mylitta* is heavily infected by bacteriosis and the pathogenic bacteria are either gram-negative micrococci or gram-positive bacilli (Jolly *et al.*, 1979). During the present study, a large number of gram-positive



FIGURES 1–4: 1. TEM of PSG showing a chain of bacilli (arrow), lysosomes (L) and vacuoles (V) during bacteriosis. X (Scale). 2. Magnified view of a single bacillus showing one broad end (arrow) and elongated flagella (F). X (Scale). Inset: Bacillus and lysosome (LY). 3. TEM of PSG showing NPV virions (arrow). X (Scale). 4. TEM of PSG showing *Nosema* spores (arrow) X (Scale).

TABLE 1. Total protein concentration in the posterior silk gland in healthy and diseased larvae of *A. mylitta*

Diseased and control larvae	Total protein concentration (μg/mg)
Bacteriosis	1.31 ± 0.21
Virosis	2.25 ± 0.31
Microsporidiosis	3.82 ± 0.63
Control	4.00 ± 0.47

rod-like bacilli forming a sort of chain were observed in the PSG cells of the larvae suffering from bacteriosis. It is worth-mentioning that, large number of lysosomes appear in close vicinity of a group of bacteria. Recent studies have elucidated release of attacins, haemolin and other lysozymes which attack and finally kill the bacteria through the process of phagocytosis (Gunne and Steiner, 1993; Stainley, 1997). On the basis of present observations, it seems quite obvious that the sub-cellular defense responses occur immediately after the bacterial infection. Phagocytosis occurs due to the process of cellular internalization of foreign invaders and the lysozymes finally induce lysis of the bacteria completely (Stainley, 1997).

This is, perhaps, the first report on ultra-structural localization of *bacilli* and subsequent occurrence of lysosome bodies in PSG cells of *A. mylitta*, suggesting the antibacterial defense mechanism. The present study shows significant reduction in total protein content of PSG after bacteriosis.

The NPV polyhydra were observed causing disease in the wild silkworms, *A. pernyi* (Tanaka, 1963) and *A. yamamai* (Kobayashi *et al.*, 1983). The present EM study revealed the presence of double rod-shaped NPV virions or capsids in the silk gland cells of *A. mylitta* larvae infected from virosis. The double rod-shaped structure of capsids however, represents the lateral walls of a capsid shell. The apical and basal end-caps are formed from altogether different peptides than the lateral walls and thus both the ends of capsids are not visible under EM (Kuzio and Faulkner, 1993). In accordance with the criteria of modern classification of viruses (Murphy *et al.*, 1995), the nomenclature of NPV of *A. mylitta* may be coined as, A_mNPV.

The microtubular radial channels in the silk gland cells were completely disappeared in *A. mylitta* larvae suffering from virosis suggesting depolymerization of microtubules due to the infection of NPV similar to that in *Spodoptera frugiperda* 1 PLB-Sf-21 cells (Volkman *et al.*, 1990).

Reduction in PSG protein content was observed significantly in the *A. mylitta* larvae infected with NPV, obviously, due to destruction of the protein secretory apparatus (Horie and Watanabe, 1980; Gururaj *et al.*, 1999).

High concentration of spores of *Nosema* in fat body, gut and Malpighian tubules was reported earlier in *A. mylitta* (Jolly and Sen, 1972; Bansal *et al.*, 1997); (Rath *et al.*, 2001) while this is, perhaps, the first report regarding *Nosema* infection in the silk gland.

In *A. mylitta*, the oval-shaped spores of *Nosema* were well evident. Hatakeyama *et al.* (2000), however, noticed that the *Nosema* isolated from *A. mylitta* represents a new strain showing different bases of rRNA from that of *N. bombycis*.

However, no significant reduction in PSG protein content was observed in *Nosema* infected *A. mylitta* larvae in comparison to that in the healthy larvae.

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Survey of medically important mosquito fauna in Mizoram

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ABSTRACT: A study was made in some places of Mizoram in two different seasons to record the mosquitoes prevalent in the state. A total of 54 species of mosquitoes under 10 genera were recorded. Forty-seven species were recorded in the monsoon season and 48 in the post-monsoon season. Both the primary vectors of malaria, *viz.*, *Anopheles dirus* and *An. minimus* were recorded. Potential vectors of dengue and Japanese encephalitis were also detected. *An. tessellatus*, *An. splendidus*, *Culex epidesmus*, *Cx. fuscocephala*, *Cx. pseudovishnui*, *Aedes aegypti*, *Ae. caecus*, *Ae. vittatus*, *Ae. nigrostriatus*, *Armigeres durhami*, *Toxorhynchites gravelyi*, *Tripteroides aranoides* and *Uranotaenia campestris* not recorded earlier from this state have been recorded in the present survey. Fifteen species reported earlier were seen missing in the present survey.

KEYWORDS: Mosquito fauna, disease vectors, malaria, Japanese encephalitis, dengue

INTRODUCTION

The Northeastern (N.E.) Region of India is having unique ecosystems that are favourable to the mosquito fauna. Many areas of NE region are still not approachable due to want of good road communication or due to difficult terrain. Hence the study on mosquitoes is very scanty (Malhotra *et al.*, 1982, 1984; Nagpal and Sharma, 1987). The present paper reports the results of a survey carried out during 1999–2000.

The survey was conducted in monsoon and post monsoon season in Aizawl, West Phaileng, Thenzawl, Kolasib, Lengpui and Champhai at altitudes ranging from 700 to 4000 ft. above mean sea level. Temperature ranged between 18–25 °C and relative humidity between 45–80%. Immatures of mosquitoes were collected from different breeding areas using standard dippers. The larvae were reared in the laboratory to get the adult mosquitoes for proper identification. Adult mosquitoes were also collected by operating CDC miniature light traps for whole night in cattle-sheds. Mosquitoes were identified using the key of Christophers (1933); Barraud (1934) and catalogue of mosquitoes by Knight and Stone (1977).

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TABLE 1. Mosquito species recorded in two different seasons in Mizoram.

Anopheles

An. (Cel.) aconitus Doenitz, 1902
An. (Cel.) annularis Van der Wulp, 1884
An. (Cel.) dirus (balabacensis) Peyton & Harrison, 1979
**An. (Cel.) jamesii* Theobald, 1901
An. (Cel.) jeyporiensis James, 1902
An. (Cel.) karwari James, 1903
An. (Cel.) kochi Doenitz, 1901
An. (Cel.) maculatus Theobald, 1901
An. (Cel.) minimus Theobald, 1903
An. (Cel.) nivipes Theobald, 1903
An. (Cel.) philippinensis Ludlow, 1902
An. (Cel.) splendidus Koidzumi, 1920
An. (Cel.) tessellatus Theobald, 1901
An. (Cel.) vagus Doenitz, 1902
**An. (Cel.) willmorei* (James), 1903
An. (Ano.) barbirostris Van der Wulp, 1884
**An. (Ano.) gigas* Giles, 1901
An. (Ano.) nigerrimus Giles, 1900

Culex

Cx. (Cux.) bitaeniorhynchus Giles, 1901
Cx. (Cux.) epidesmus (Theobald), 1910
Cx. (Cux.) fuscocephala Theobald, 1907
Cx. (Cux.) gelidus Theobald, 1901
Cx. (Cux.) mimeticus Noe, 1899
Cx. (Cux.) mimulus Edwards, 1915
Cx. (Cux.) pseudovishnui Colless, 1957
Cx. (Cux.) quinquefasciatus Say, 1823
Cx. (Cux.) tritaeniorhynchus Giles, 1901
Cx. (Cux.) vishnui Theobald, 1902
Cx. (Cux.) whitmorei Giles, 1904
***Cx. (Cui.) pallidothorax* Theobald, 1905
***Cx. (Eum.) brevipalpis* (Giles), 1902
Cx. (Lut.) fuscanus Wiedemann, 1920

Aedes

***Ae. (Stg.) aegypti* (Linnaeus), 1762
Ae. (Stg.) albopictus (Skuse), 1894
Ae. (Stg.) annandalei Theobald, 1910
***Ae. (Stg.) vittatus* Bigot, 1861
**Ae. (Aed.) caecus* (Theobald), 1901
**Ae. (Aed.) nigrostriatus*(Barraud),1927
Ae. (Fin.) albolateralis (Theobald), 1908
Ae. (Fin.) pseudotaeniatus (Meigan), 1830
Ae. (Aed.) vexans (Giles), 1901
Ae. (Fin.) chrysolineatus (Theobald), 1907

Mansonia

Ma. (Mno.) annulifera (Theobald), 1901
Ma. (Mno.) indiana Edwards, 1930
Ma. (Mno.) uniformis (Theobald), 1901

Armigeres

***Ar. (Arm.) durhami* Edwards, 1917
Ar. (Arm.) kuchingensis Edwards, 1915
Ar. (Arm.) subalbatus (Cuquillett), 1898

Malaya

Ml. (Mao.) genurostris Leicester, 1922

Toxorhynchites

Tx. (Tox.) splendens (Wiedemann), 1919
**Tx .(Tox.) gravelyi* (Edwards), 1921

Coquillettidia

Cq. (Coq.) crassipes (Van der Wulp), 1991

Tripteroides

Tp. (Rah.) aranoides (Theobald), 1901

Uranotaenia

***Ur. (Ura.) campestris* Leicester, 1908

* Present in monsoon season only.
** Present in post-monsoon season only.
Rest present in both the seasons.

Fifty-four species of mosquitoes under 10 genera were recorded (Table 1). In monsoon season, 47 species and in post monsoon season, 48 species were collected. The major malaria vectors of the northeast, *Anopheles minimus* was collected from Thenzawl area which is a valley surrounded by hills, broken forests and traversed by

different types of streams, facilitating the profuse breeding of vector mosquito. The other vector, *An. dirus* was collected from West Phaileng area which is surrounded by forests (including teak plantations). *An. minimus* and *An. balabacensis balabacensis* Baisas (*An. dirus*) were incriminated as vectors of malaria from Mizoram (Das and Baruah, 1985; Das *et al.*, 1990).

Eighteen species of *Anopheles* were collected. *An. tessellatus* and *An. splendidus* were first reported from Mizoram. *An. culicifacies*, *An. dthali*, *An. majidi*, *An. pseudojamesii*, *An. subpictus* and *An. theobaldi* reported in earlier studies (Malhotra *et al.*, 1982, 1984; Nagpal and Sharma, 1987) were not recorded in areas in Mizoram covered in the present study.

Among Culicines, nine genera *viz.*, *Culex*, *Aedes*, *Mansonia*, *Malaya*, *Armigeres*, *Coquillettidia*, *Toxorhynchites*, *Tripteroides* and *Uranotaenia* have been detected in the present study comprising 14 species of *Culex*, 10 species of *Aedes*, 3 species of *Mansonia*, 3 species of *Armigeres*, 2 species of *Toxorhynchites*, and one species each of *Tripteroides, Uranotaenia, Coquillettidia* and *Malaya* (Table I). It may be seen from the table that in monsoon season, *Aedes aegypti*, *Ae. vittatus*, *Ae. nigrostriatus*, *Culex pallidothorax*, *Cx. brevipalpis*, *Armigeres durhami*, *Uranotaenia campestris* and in post monsoon season, *Aedes caecus* and *Toxorhynchites gravelyi* have not been detected. *Culex epidesmus*, *Cx. fuscocephala*, *Cx. pseudovishnui*, *Aedes aegypti*, *Ae. caecus*, *Ae. vittatus*, *Ae. nigrostriatus*, *Armigeres durhami*, *Toxorhynchites gravelyi*, *Tripteroides aranoides*, *Uranotaenia campestris* were not reported earlier. *Culex cornutus*, *Cx. sinensis*, *Cx. viridiventer*, *Cx. malayi*, *Mimomyia chamberlaini*, *Aedes novoniveus*, *Ae. poecilus*, *Armigeres magnus* reported in earlier surveys (Malhotra *et al.*, 1982, 1984; Nagpal and Sharma, 1987) were not recorded in the present study.

Among the proven vectors of Japanese encephalitis virus transmission in India (Pant, 1979), most of the vectors *viz.*, *Culex epidesmus*, *Cx. vishnui*, *Cx. pseudovishnui*, *Cx. tritaeniorhynchus*, *Cx. gelidus*, *Cx. whitmorei*, *Cx. bitaeniorhynchus*, *Cx. fuscocephala*, *Mansonia annulifera* have been recorded from Mizoram.

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Frass mediated host finding behaviour in *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae), a larval parasitoid of *Chilo partellus*

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ABSTRACT: Frass mediated host finding behaviour of *Cotesia flavipes* was studied utilizing a T-tube olfactometer. In a no-choice situation *C. flavipes* females were attracted strongly to the arm containing the larval frass of *Chilo partellus*. Per cent attraction to the larval frass obtained through feeding different host plants ranged from 75 to 64 % and these were not significantly different from all getting attracted to the frass containing arm. Attraction to larval washings was just 35% and significantly different from all getting attracted to the arm. In a paired-choice test, larval frass obtained from feeding on maize was most attractive followed by sorghum and bajra.

KEYWORDS: *Chilo partellus, Cotesia flavipes,* frass, host finding, larval parasitoid

Lepidopteran stem borers are considered to be the most damaging to maize. Losses due to stem borers to the extent of 23–100 per cent have been reported in maize (Seshu Reddy and Walker, 1990). The most important stem borer species in India is *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae). *C. partellus* is parasitised by many larval and pupal parasitoids (Sharma *et al.*, 1966) of which the larval parasitoid, *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) is important under field conditions (Mohan *et al.*, 1991; Devi and Raj, 1996).

The use of volatile chemical cues in host microhabitat location is well known in parasitoids (Vet and Dicke, 1992). Kajitha and Drake (1969) and Mohyuddin (1971) reported attraction of females of *C. flavipes* to the larval frass of *C. partellus.* Studies have shown that frass acts as a direct mediator in the host finding behavior

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of *C. flavipes* (Leerdam Van *et al.*, 1985; Potting *et al.*, 1995; Ngi-Song *et al.*, 1996). *C. partellus* feeds on maize, sorghum, ragi, bajra, etc. and the differential attraction, if any, of the parasitoid to the frass from these host plants assumes significance. A comparison of the attraction of *C. flavipes* to larval frass of *C. partellus* fed on different graminaceous plants is presented.

The initial culture of *C. flavipes* was started from naturally parasitised *C. partellus* larvae collected from maize fields in and around Bangalore. *C. partellus* larvae were reared on artificial diet in the laboratory and utilized for rearing the parasitoid. After parasitisation, *C. partellus* larvae were allowed to feed on the diet at 24 °C and 65–70 per cent humidity. Parasitoid cocoons were collected in glass vials and kept in a clean cage until emergence. After emergence adult parasitoids were provided a 50% honey/water solution. Parasitoids used in the experiments were one-day-old naive, mated females.

C. partellus larvae from the laboratory culture were fed on fresh stems of maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L.), bajra (*Pennisetum typhoides* L.) and ragi (*Eleusine coracana* L.) and frass produced for the first 24 h period discarded. Frass produced during the following 24–48 h was collected. Larvae were also fed on freshly prepared artificial diet and the frass collected similarly. One gram of fresh frass obtained from the four plant species and artificial diet were used for the tests. To obtain larval washings five *C. partellus* larvae were put into 5 ml distilled water, thoroughly washed and removed. One ml of this washing soaked in cotton was used for the test.

A T-tube olfactometer was constructed from two acrylic (non-absorbent, odourless and transparent) tubes of 2 cm diameter (ID), joined to form a 'T'. The arms were 10 cm long and the stem 15 cm long. The stem of the 'T' was connected to a 10 cm diameter funnel (Fig. 1). Olfactometer tests were conducted by placing the frass or cotton in small vials (3 × 2.5 cm) behind a mesh and the vials placed tightly into arms of the T-tube (Fig. 1). The olfactometer was covered with a paper box and uniform light source provided from the top. Tests were conducted at 25–27 °C and 65–75% relative humidity. Ten parasitoids were released into the olfactometer through the wide part of the funnel and the arm chosen (an arm was considered chosen when the parasitoid reached the end of the arm) by the test insects recorded at the end of 15 minutes. At the end of each test, the test population was changed and the olfactometer washed and shade dried. In the no-choice test in one arm the frass/larval washing was placed while the other arm was left empty. The number of females attracted to the arm containing frass was taken as the observed frequency and tested using a χ^2-test with the expected frequency of all released females moving to the arm. Each test was replicated five times. In the paired-choice test, frass from all the five host plants were tested in pairs (ten pairs in all) by placing them simultaneously at the end of each arm. Each pair was replicated five times and the mean number of females attracted to the two arms compared (t-test).

It was generally observed that the parasitoids climbed the stem of the T-tube and moved towards one of the two arms. In a no-choice situation, *C. flavipes* females were attracted only to the arm with frass, regardless of the plant species upon which

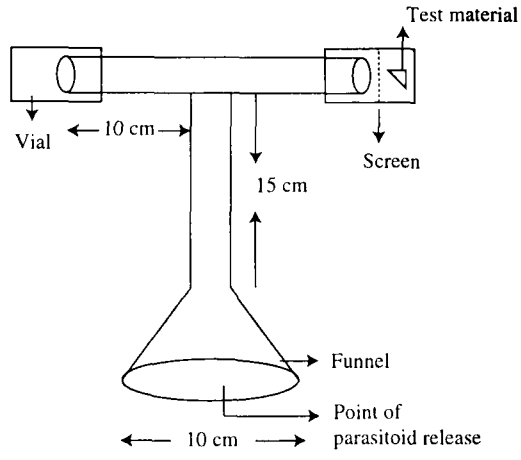

FIGURE 1. A schematic diagram of the T-tube olfactometer used for the tests.

C. partellus larvae had fed, as compared to the empty arm. Per cent attraction of the released females to the frass of different plants was 75% for maize, 72% for sorghum, 70% for bajra, 68% for artificial diet and 64% for ragi. The observed attractions were not significantly different from all getting attracted to the frass-containing arm (χ^2 test, $P \leq 0.01$). Larval washings placed in the arm attracted just 35% of the females and was not significantly different from all getting attracted to the arm (χ^2 test, $P \leq 0.01$). Leerdam Van *et al.* (1985) found the same kind of attraction by *C. flavipes* to the frass of sugarcane and Johnson grass in a no-choice situation. The results clearly demonstrate that volatiles present in the frass mediate the host finding behaviour of *C. flavipes* and confirm the results of Inayatulla (1983) and Potting and Overholt (1997).

The paired-choice test revealed that as compared to frass from sorghum, bajra, ragi and artificial diet, maize was the most attractive (Table 1). Sorghum frass was more attractive than frass from all other hosts but maize. Frass from bajra was more attractive than frass from ragi and artificial diet but less attractive than maize and sorghum. Frass from ragi and artificial diet were equal in their attraction. These findings are in consonance with the results of Inayatulla (1983) who found that maize and sorghum were highly attractive and sugarcane least attractive. Leerdam Van *et al.* (1985) found that when a choice was given, sugarcane was more attractive than Johnson grass. In a T-tube olfactometer study, Ngi-Song and Overholt (1997) found that *C. flavipes* and *C. sesaminae* were not able to discriminate among frass of four different host plants tested.

It is evident from the strong attraction of *C. flavipes* to the frass of *C. partellus* that volatiles present in the frass are mediating the host finding behavior of *C. flavipes*. In a no-choice situation *C. flavipes* is equally attracted to the frass irrespective of its plant origin and in a choice situation, attraction depended on the plant species on which the host fed, being highest for maize. It would be worthwhile to identify the volatile

TABLE 1. Attraction of *Cotesia flavipes* to larval frass of *C. partellus* from different host plants in an olfactometer paired-choice test

Paired frass of	Mean number of females attracted to the arm with		*t*-value
	Frass from host plant 1 in pair	Frass from host plant 2 in pair	
Maize–Sorghum	6.8	**2.4**	6.95**
Maize–Bajra	5.6	**4.0**	3.13*
Maize–Ragi	6.4	**3.0**	5.01**
Maize–Diet	5.8	**2.8**	5.66**
Sorghum–Bajra	5.2	**3.2**	3.77**
Sorghum–Rabi	5.8	**3.0**	5.66**
Sorghum–Diet	5.4	**3.0**	4.00**
Bajra–Ragi	5.8	**2.8**	5.66**
Bajra–Diet	5.2	**1.8**	4.90**
Ragi–Diet	4.6	4.4	0.3 NS

** Significant at $P \leq 0.01$. * Significant at $P \leq 0.05$. NS–Not significant.

chemicals from the frass of suitable host plants like maize and sorghum to understand and improve the parasitising efficiency of *C. flavipes* on *C. partellus*.

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The life cycle, ecological role and biology of immature stages of *Heliocopris dominus* Bates (Coleoptera: Scarabaeidae: Coprinae)

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ABSTRACT: *Heliocopris dominus* lays 4 eggs, each being enclosed in a egg pouch which is eccentrically located in a brood ball made from fresh elephant dung. The egg of *H. dominus* appears to be the largest of insectan eggs yet recorded. The larvae through 3 instars, the first and second instars last two months each. The prolonged development of the third instar takes place in two stages—an actively feeding stage lasting 3 and half months during the later half of the wet season and an aestivating, quiescent stage during the first three months of the dry season. The pupae complete their transformation by the end of the dry season. The adults emerge early in June with the advent of the heavy rains of the South–West Monsoons. There is only one annual generation. As the major agency for the removal and utilization of elephant dung during the rainy season in forests, the *Heliocopris* beetles play an important role in the proper functioning of their ecosystem.

KEYWORDS: *Heliocopris dominus*, largest insectan egg, immature stages, life cycle

Field work for the present paper was carried out in the Karulai Forests, Nilambur Subdivision, Kerala, from June 1989 to July 1991 (Joseph, 1991, 1994, 1998).

BIOLOGY OF IMMATURE STAGES AND THE LIFE-CYCLE

(a) *The egg*. The newly laid egg (Fig. 1) is pale yellowish-white, cucumber-shaped, with a tough, opaque and glistening chorion. The egg is located inside an egg-pouch (length: 52–55 mm (53.80 ± 1.30; $n = 5$); width: 43–45 mm (43.60 ± 0.89; $n = 5$); height: 28–30 mm (28.80 ± 0.84; $n = 5$). It is made of fresh elephant dung and is lined with a thin layer of soil which serves to protect the egg from direct contact with dung and so possibly helps to preclude fungal attack. Inside the egg-pouch the egg remains in a slanting position with its anterior (head) end slightly raised and loosely fixed to the wall of the pouch by means of a cementing secretion of the mother's colleterial glands (Hinton, 1981). Five such eggs were measured and weighed. Their length varied from 21.50–22 mm (21.75 ± 0.25; $n = 5$); diameter in the middle varied from 9.80–10 mm (9.92 ± 0.8; $n = 5$); weight varied from 0.92–0.95 g (0.93 ± 0.11; $n = 5$).

According to Hinton (1981), some of the eggs with the greatest volume are those of beetles, e.g., the 130 mm long female cerambycid *Titanus giganteus* (L.) has an egg size of: length 10.12 mm × diameter 4.2 mm, and the 45 mm long buprestid of the genus *Sternocera* has an egg size of: length 7.3 mm × diameter 5.2 mm. Kingston and Coe (1977) found that the egg of *Heliocopris dilloni* is 19–20 mm in length, 7 mm in diameter and weighed 0.71 g (mean 0.63 g). Thus the egg of *H. dominus* described above is indeed the largest of the known insectan eggs yet recorded.

Eggs older than 24 h after laying develop a yellowish coloration which deepens with age. The eggs of *H. dominus* could be found in nests from the beginning of the 1st week of July to the end of the last week of August.

(b) Larval instars

(i) The first instar larva hatches on the seventh day after the egg is laid. The newly hatched larvae (Fig. 2) are pale yellowish and weighed from 0.89–0.90 g (0.894 ± 0.005; $n = 5$). This larva has a head capsule width of 6.00 mm. It started feeding only two days later when its head capsule and spiracles hardened and darkened. After 6 days of feeding and growth, the larvae weighed from 3.05–3.20 g (3.13 ± 0.06; $n = 5$). The first instar larvae could be found in the field from the beginning of the second week of July to the end of the first week of September.

(ii) The second instar larva (Fig. 3) has a head capsule width of 9 mm. This stadium lasts for 15 days, during which period it feeds actively and grows, takes on a pale brownish-grey coloration. The larvae weigh from 8.95–9.15 g (9.06 ± 0.09; $n = 5$). The second instar larvae were found in the field from the beginning of the third week of July to the end of the third week of September.

(iii) The third instar larvae have a head capsule width of 13 mm. At the beginning of this stadium, the larvae weigh from 12.85–13.20 g (13.04 ± 0.16; $n = 5$). The actively feeding third instar larvae were met with in the field from the beginning of the second week of August to the end of December. This larva is a voracious feeder in which the body is doubled up on itself in the form of the letter '*V*' and is distinctly hump-backed (Fig. 4). The doubling up of the body begins in the first instar, increases in the second instar, and is at its maximum in the late third instar. Already by the third week of November (at about 95 days of age), these brownish-grey larvae had attained a giant size and weighed from 41.55–43.67 g (42.65 ± 1.01; $n = 5$) with still more of the provisioned dung remaining to be consumed. These larvae continued to feed for a further period of 30 days and by the end of December attained a maximum weight varying from 46.40–47.53 g (47.11 ± 0.51; $n = 5$) at around 4 months after the end of the egg-laying period of the species.

After the completion of feeding and attaining maximum growth coinciding with the onset of the dry season from the beginning of January onwards, the third instar larvae enter into a *quiescent state* and aestivate inside their brood balls for a period of about 3 months from January to March. During this period, these larvae lose weight

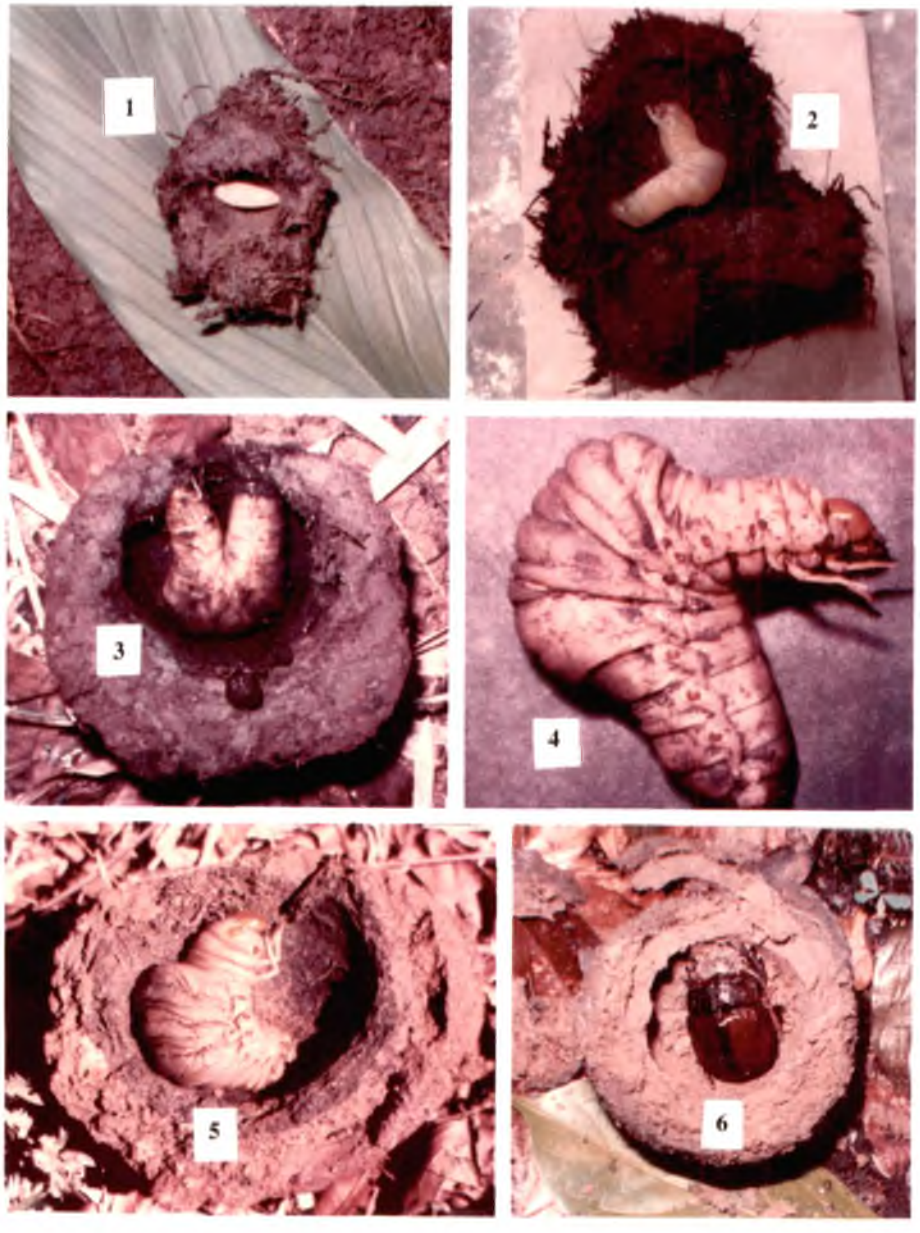

FIGURE1–6: 1. The newly laid egg in the opened out egg-pouch; 2. The newly hatched first instar larva in the opened out egg-pouch; 3. The actively feeding second instar larva in its brood ball; 4. The third instar larva which has almost completed feeding, removed from its brood ball; 5. The aestivating stage of the third instar larva inside the spacious cavity of its brood ball; 6. The newly enclosed adult (teneral form), its head still ensheathed by a portion of the pupal cuticle, remaining quiescent inside its opened brood ball.

by voiding their gut contents on to the wall of the cavity of their brood balls. Before pupation beginning during the last week of March, their weight varied from 28–32 g (30.19 ± 1.82; $n = 5$) (Fig. 5).

(c) The pupa

The pale-yellowish early pupae were found in marked nests in the field from the 4th week of March to the end of the 1st week of May. The late pupal stage takes on a more yellowish coloration before it turns reddish-brown prior to its final metamorphosis into the adult. The reddish-brown pupae were observed in the field from the beginning of the 2nd week to the end of the 4th week of May.

(d) The teneral adult

The adult form that has just enclosed from its pupal cuticle, remains motionless inside its brood ball for about 3 days until its soft cuticle hardens and takes on the dark brownish-black adult coloration. The teneral adults (Fig. 6) were seen in nests from the last week of May to the end of the 1st week of June.

(e) the adults

are forced to remain inside their intact brood balls until sufficient rain water penetrates into their brood chamber and the brood balls become water-soaked. Then each beetle cuts out a hole of diameter form 3.5–4 cm on the upper wall of its brood ball and escapes into the common brood chamber of the nest. Later these beetles tunnel through the moist soil layer above their brood chamber and reach the surface of the top soil, as evidenced by the presence of 3 emergence holes (3.5–4 cm in diameter) right above the brood chambers of 4 marked nests.

In the year 1991, in the Karulai Forest area of Nilambur, the emergence of adults of *H. dominus* occurred from the 1st week of June to the end of July (Joseph, 1998). It is concluded from this study of the life-cycle that *H. dominus* is *univoltine*, with only one annual brood.

ECOLOGICAL ROLE

In many areas of the natural distribution of elephants in Africa and Asia, the *Heliocopris* beetles constitute the most important agency of elephant dung removal and its utilisation during the rainy season. Kingston and Coe (1977) recorded up to 60 individuals of *H. dilloni* at their peak emergence period on a single pat of fresh dung of the African elephant. Coe (1972, 1977) found that a single individual of *H. dilloni* buried about 40% of the material of one elephant dung pat of mean weight of 7.4 kg. In the Karulai Forests during the present study, from 4–7 individuals of *H. dominus* (during their peak emergence period) were observed on a single pat of fresh elephant dung and that a single individual of *H. dominus* removed up to 42% of the material of one dung pat of mean weight of 5.850 kg.

Besides utilising the dung removed for making brood balls, the adult beetles of both sexes feed on dung from the time of their emergence (during maturation feeding) and throughout their breeding period. The quantities of dung removed and utilised by these beetles can thus be enormous.

Through their habit of tunneling in the soil, often descending to considerable depths (30–120 cm in *H. dilloni*, Kingston and Coe (1977) and 27–56 cm in *H. dominus*, Joseph (1998), these beetles help in increasing its aeration and water percolation capacity. By bringing up the deeper layers of the soil from their excavations, they recycle materials, adding to fertility. More importantly, they bring about natural manuring in the forest: the wet dung these beetles carry down to the deeper layers of the soil, undergoes rapid decomposition, releasing nutrients for immediate mobilisation for the growth of forest trees and plants (Coe, 1988). The *Heliocopris* beetles thus contribute to the proper functioning of their ecosystem.

The idea for taking up research on the *Heliocopris* beetles occurred to the author while attending a talk in 1947 by the late distinguished Professor Dr. K. S. Padmanabha Iyer at the Maharaja's College, Trivandrum. This work was financed by a grant from the STEC, Government of Kerala, for which the author is highly indebted. He thanks the authorities of the Kerala Agricultural University and of the College of Horticulture, Vellanikkara, Trichur, for facilities for work and for various courtesies.

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Ultrastructural studies on mouthparts of four species of genus *Culex* Linnaeus (Diptera: Culicidae)

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ABSTRACT: The mouthparcs of four species of female mosquitoes belonging to genus *Culex* Linnaeus *viz.*, *Culex* (*Culex*) *quinquefasciatus* Say, two species of *Culex* (*Culex*) *vishnui* subgroup, *Culex* (*Culex*) *vishnui* Theobald and *Culex* (*Culex*) *tritaeniorhynchus* Giles, one species of *Culex* (*Culex*) *bitaeniorhynchus* subgroup, *Culex* (*Culex*) *bitaeniorhynchus* Giles, have been scanned under an electron microscope. The number, shape, and size of teeth and sensilla on the maxillary stylets and palps, and finger like cuticular projections on the hypopharynx have been found to be extremely useful in separating these closely related species which are otherwise difficult to identify.

KEYWORDS: Culicidae, *Culex* spp., scanning electron microscopy, mouthparts, Japanese encephalitis

INTRODUCTION

Japanese encephalitis (JE), first recorded in Tamil Nadu in 1955 (Chakraborty, 1990), has now become a serious public health problem in almost all parts of this country. Among other genera of mosquitoes, species of *Culex* (*Culex*) Linnaeus are the major vectors of this disease. The identification of the adult mosquitoes of *Culex* spp. has been found to be extremely difficult. The fauna of British India (Barraud, 1934) and monographs by Bram (1967) and Sirivanakarn (1976) include descriptions based largely on body colouration and size of different parts which are too variable to rely upon. Reuben (1969) also stressed the colouration of the legs for separating the component species of the *Culex vishnui* group. Even the illustrated keys to species of *Culex* (*Culex*) (Reuben *et al.*, 1994) are based on the colouration of legs which also show morphological variations. There is thus an urgent need to search for more stable supplementary taxonomic characters from other fields, including electron microscopy, for the characterization of such an important group of mosquitoes. An attempt has

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been made to study the detailed structure of the female mouthparts of four culicine species *viz.*, *Culex quinquefasciatus*, *Culex tritaeniorhynchus*, *Culex bitaeniorhynchus* and *Cx. vishnui* under the scanning electron microscope and to use these features as reliable discriminatory characters to supplement the available taxonomic characters. The present four species of mosquitoes were collected from human dwellings as well as mixed dwellings *i.e.*, rice field, cattle sheds and walls of houses of different districts of Punjab (India) and identified from Regional office of H & W (NICD) Chandigarh.

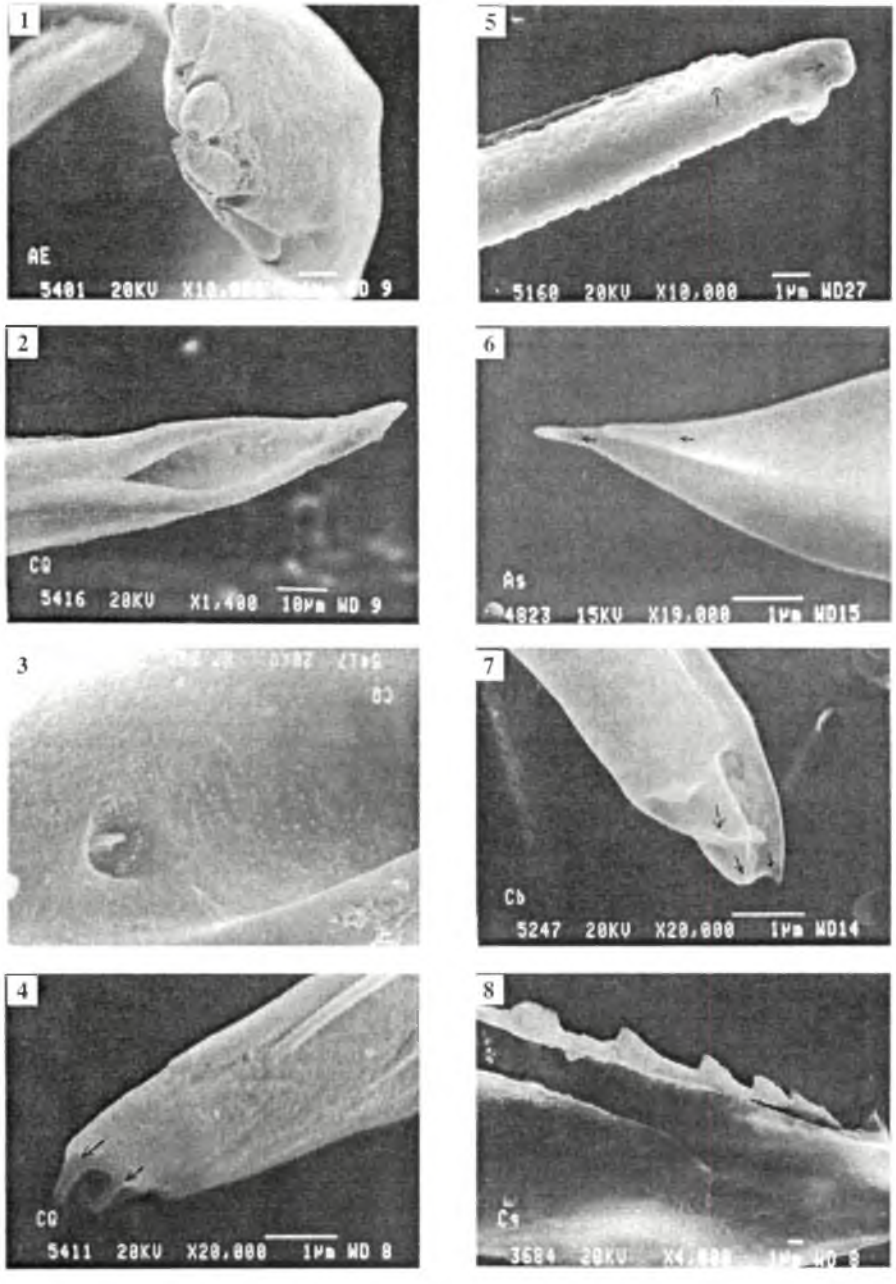
For SEM studies, the samples were fixed in 3% gluteraldehyde, dehydrated in graded series of alcohol and mounted on SEM specimen stubs using only a small strip of double-side adhesive tape (in order to avoid their penetration into the tape). The samples were then sputter coated with gold and scanned under JSM-6100 scanning electron microscope. For each species, five specimens were examined. The terminology for various structures and sensilla has been adopted from Mclver and Charlton (1970) and Lee and Craig (1983a,b).

The studied mouth parts include the labrum, mandibles, maxillae and the hypopharynx. Different types of sensilla and teeth on the mouth parts of four female culicine mosquitoes are described below.

The labrum-epipharynx occupies the most anterior position. It consists of a large ventral epipharyngeal surface and a small dorsal surface. The labrum bears two pairs of sensilla, a pair at its tip and a subapical pair along its lateral margins (Fig. I). In addition, a campaniform sensillum lying near the proximal end of the epipharyngeal surface has been observed only in *Cx. quinquefasciatus* (Figs 2 and 3).

Out of four species included in this study, a campaniform sensillum has been witnessed only in *Cx. quinquefasciatus* Say and is missing in the remaining three species. Froelich (1971) also recorded such a sensillum in *Culex pipiens* and *Culex pipiens fatigans* respectively, the same taxon. It thus appears that the presence of a campaniform sensillum is a common phenomenon in the *Cx. quinquefasciatus* and thus provides a very convincing evidence for the identification of *Cx. quinquefasciatus*. In addition to this, a pair of such sensilla has been observed in *Aedes aegypti* (Lee, 1974). The paired apical and subapical sensilla noted in the present four species of *Culex* have also been recorded in *Cx. pipiens fatigans* (Froelich, 1971). In fact, paired apical and subapical sensilla of labrum have also been reported in two other genera *viz.*, *Anopheles* (Lee and Craig, 1983a,b), and *Aedes* (Hudson, 1970; Lee, 1974). The constant presence and location of paired apical and subapical sensilla on the labrum appears to be a characteristic feature of Culicidae and, therefore, not of any taxonomic significance for distinguishing genera or their component species.

The hypopharynx is a flat stylet lying between the mandibles and maxillae. The pointed apex of the hypopharynx bears four finger-like processes in *Cx. quinquefasciatus* (Fig. 4) which are absent in the *Cx. vishnui* group of mosquitoes. In this group, *Cx. tritaeniorhynchus* has the surface of the hypopharynx quite rough (Fig. 5), while in *Cx. vishnui* the surface of the hypopharynx is smooth (Fig. 6). *Cx. bitaeniorhynchus* has the tip of the hypopharynx clearly bifid, with one end blunt and the other pointed (Fig. 7). Another characteristic feature of *Cx. quinquefasciatus* relates to the presence

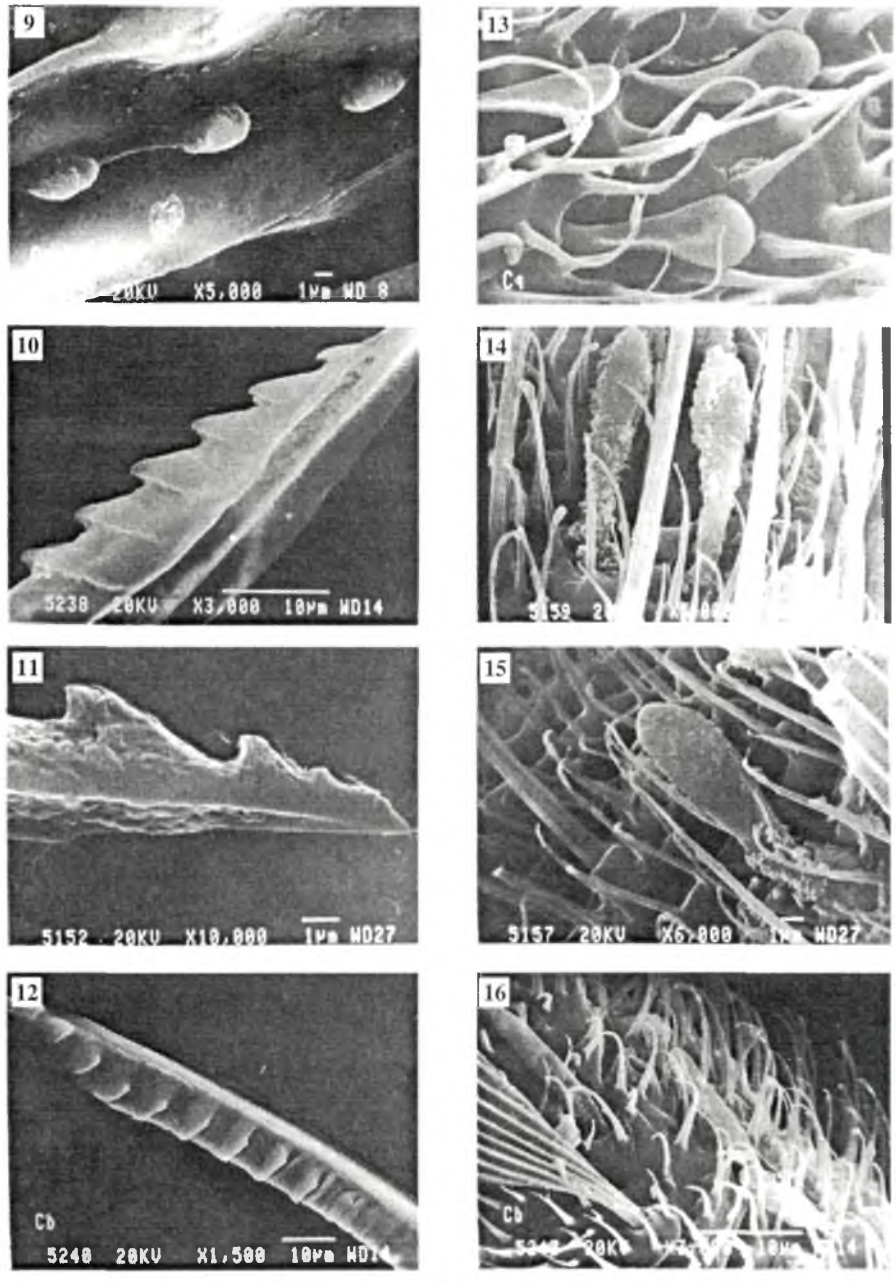


FIGURES 1–8: 1. Labrum (Dorsal view). 2. Labrum (Ventral view). 3. Campaniform sensilla on the labrum. 4. Tip of hypopharynx of *Cx. quinquefasciatus*. 5. Tip of hypopharynx of *Cx. tritaeniorhynchus*. 6. Tip of hypopharynx of *Cx. vishnui*. 7. Tip of hypopharynx of *Cx. bitaeniorhynchus*. 8. Maxillary stylet (pointed teeth) of *Cx. quinquefasciatus*.

of 4 finger-like processes at the tip of the hypopharynx as these are missing in the remaining three species studied. Lee and Craig (1983a), on the contrary, reported the presence of 8–9 such finger-like processes in five species of genus *Culex* including *Cx. tritaeniorhynchus*, which has been found without these processes in the present study. Moreover, they also found 8–9 processes in four species of *Anopheles* and 13 species belonging to other genera. Earlier, Hudson (1970); Lee (1974) had also observed the same number of processes in three species belonging to genus *Aedes*. It follows from the above observations that the presence of 8–9 finger-like processes on the hypopharynx is a common feature among different genera of Culicidae. Their noted absence in three species of *Culex* and presence of only 4 processes in *Cx. quinquefasciatus* are exceptions. However, other characters of the hypopharynx can be used for characterizing the component species of the *vishnui* complex.

The maxillae of mosquitoes are highly modified, each consisting of a five-segmented maxillary palp and an elongated lacinia forming a stylet. The outer surface of each stylet is armed in its distal part with posteriorly directed teeth that are usually referred to as lateral teeth. Their number, shape, and size are different in all the four species. *Cx. quinquefasciatus* carries 13 teeth out of which the anterior seven teeth are pointed and the remaining six rounded (Figs 8 and 9). In *Cx. tritaeniorhynchus*, all the 13 teeth are blunt (Fig. 10). The number of teeth is 11 in *Cx. vishnui* but these have rough surface (Fig. 11). In *Cx. bitaeniorhynchus*, the number of teeth is again 11 and their shape is different from those of other species (Fig. 12). The maxillary palps, being supplementary sensory structures, carry four different kinds of structures, namely scales, microtrichia, sensilla chaetica, and capitate pegs or bulb-shaped sensilla. The capitate pegs are club-shaped in two species with their surface quite smooth in *Cx. quinquefasciatus* (Fig. 13) and very rough in *Cx. tritaeniorhynchus* (Fig. 14). In *Cx. vishnui*, the peg is enormously swollen at its tip (Fig. 15). In *Cx. bitaeniorhynchus*, the capitate pegs are both club-shaped and rod-like (Fig. 16). The surface of the club-shaped sensilla is rough while that of rod-shaped pegs is smooth. The length of these pegs also varies to a great extent.

The results of the present study and a review of the relevant literature reveal that the lateral teeth on the outer margin of the lacinia are present in all species of *Culex* and other genera so far studied for this purpose. As far as genus *Culex* is concerned, the number of lateral teeth is 11 in *Cx. bitaeniorhynchus* and *Cx. vishnui* and 13 in *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus*. Moreover, the number combined with the shape and texture of the teeth proves quite helpful in the discrimination of the four species under reference. The other species of this genus studied by Lee and Craig (1983b) show a variable number of lateral lacinal teeth ranging between 12 and 15. Among other genera of Culicidae, the number of lateral teeth is a minimum of 2 in genera *Wyeomyia* and *Culiseta* (Hudson, 1970; Lee and Craig, 1983b) and a maximum of 23 in genus *Aedes* (Lee and Craig, 1983b). Their number and texture appear to be distinct in different taxa. The four types of structures noticed *i.e.,* scales, sensilla chaetica, microtrichia and bulb-shaped sensilla on the surface of maxillary palps in the present four *Culex* species have also been reported in four other species of this genus,



FIGURES 9–16: 9. Maxillary stylet (rounded teeth) of *Cx. quinquefasciatus*. 10. Maxillary stylet (blunt teeth) of *Cx. tritaeniorhynchus*. 11. Maxillary stylet of *Cx. vishnui*. 12. Maxillary stylet of *Cx. bitaeniorhynchus*. 13. Capitate pegs of *Cx. quinquefasciatus*. 14. Capitate pegs of *Cx. tritaeniorhynchus*. 15. Capitate pegs of *Cx. vishnui*. 16. Capitate pegs of *Cx. bitaeniorhynchus*.

TABLE 1. Sensory structures and teeth on the mouthparts of four female culicine mosquitoes

	C. (C) quinquefasciatus Say	*C. (C) tritaeniorhynchus* Giles	*C. (C) bitaeniorhynchus* Giles	*C. (C) vishnui* Theobald.
Laburm	A single campani form sensillum near the proximal end of epipharyngeal surface	Absent	Absent	Absent
Hypopharynx	Four finger-like processes at the tip	Processes are absent but the whole surface of the hypopharynx is rough	Processes are absent, but the tip is bifid having one end blunt and other pointed	Processes are absent and surface of the hypopharynx is smooth
Maxillae	Lateral teeth 13, 7 pointed and 6 rounded	Lateral teeth 13, all blunt	Lateral teeth 11, somewhat pointed	Lateral teeth 11, with very rough surface
Maxillary Palps	The capitate pegs fully club-shaped and their mean length is 10.3 μm	Capitate pegs club shaped with very rough surface and their mean length is 13.4 μm	Capitate pegs both club-shaped and rod-shaped, former with a very rough surface. Their mean length is 14.5 μm	Capitate pegs club-shaped and each much swollen at its tip. Their mean length is 14.0 μm

two species of *Aedes* (Mclver and Charlton, 1970; Mclver, 1971), and three species of *Anopheles* (Mclver and Siemicki, 1975). This characteristic therefore appears to be a common feature of mosquitoes. The bulb-shaped sensilla in the studied species show a length of 10.3–14 μm as against the length range of 16–18 μm in other species of this genus (Mclver and Charlton, 1970). However, the shortest length of 10.3 μm in *Cx. quinquefasciatus* along with its very smooth surface go to distinguishing this species from the other three species of *Culex* under study.

In females, the mandibles are situated immediately below the labrum. In culicine mosquitoes, mandibular teeth are absent. They have been reported only in anopheline mosquitoes (Robinson, 1939). The position of various structures on the mouth parts of the four *Culex* species studied are shown in Table 1.

It is thus evident from the foregoing discussion that the sensilla present on the labrum, the hypopharyngeal processes, the lateral teeth of lacinia, and the shape and texture of the bulb-shaped sensilla and lacinial teeth can be profitably utilized for the separation of the four species of *Culex*, which are not only of economic importance but also belong to two subgroups of *Cx. vishnui* complex. Moreover, the same features of the mouth parts of mosquitoes can be used to a much larger extent for the discrimination of different species of *Culex* and to a limited extent for the characterization of species of other genera of Culicidae.

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Short Communication No. ent.28311

A new report of *Cerataphis palmae* (Ghesquire) = *C. variabilis* Hille Ris Lambus (Homoptera: Aphididae: Hormaphidinae) as pest of *Calamus dransfieldii*

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ABSTRACT: *Cerataphis palmae* (Ghesquire) (Insecta: Aphididae) is recorded as a pest on the cane, *Calamus dransfieldii* Renuka. *Calamus rotang* was also found attacked, but at lower severity.

KEYWORDS: *Calamus dransfieldii*, *Cerataphis palmae*, aphid

Calamus dransfieldii Renuka (Arecaceae: Calamoideae) is a solitary moderate sized cane endemic to Dhoni hills of Palakkad District in Kerala (Renuka, 1999). Attempts are being made to cultivate this cane with a view to conserve the species. In one plot at Palappilly, (KFRI Field Station) the canes were seen infested by aphids damaging inflorescence during the flowering season in November–December 2001. The infested inflorescence dried up subsequently (Fig. 1). The aphids collected from the cane were identified as *Cerataphis palmae*. The pest was effectively controlled by spraying the insecticide Endosulfan 35EC at 0.2% a.i. No other major pests were observed in *C. dransfieldii*. The inflorescence of another species of cane, *Calamus rotang* also was found attacked by this aphid in the KFRI campus, Peechi, in October 2001. However, the severity of damage was low.

David (1958) recorded *C. variabilis* (= *C. lantaniae*) as infesting *Calamus* rotang from Coimbatore, Tamil Nadu. However, he did not find them as injurious to the host plants. Other recorded hosts of *C. variabilis* include *Areca catechu*, *Elaeis guineensis* and *Lantana aculeata* (Beeson, 1941). *C. palmae* is also recorded as a pest of coconut from India (Rajagopal *et al.*, 1990; Nayar *et al.*, 1976) and Nigeria (Enobakhare and Omogiate, 2000).

*Corresponding author

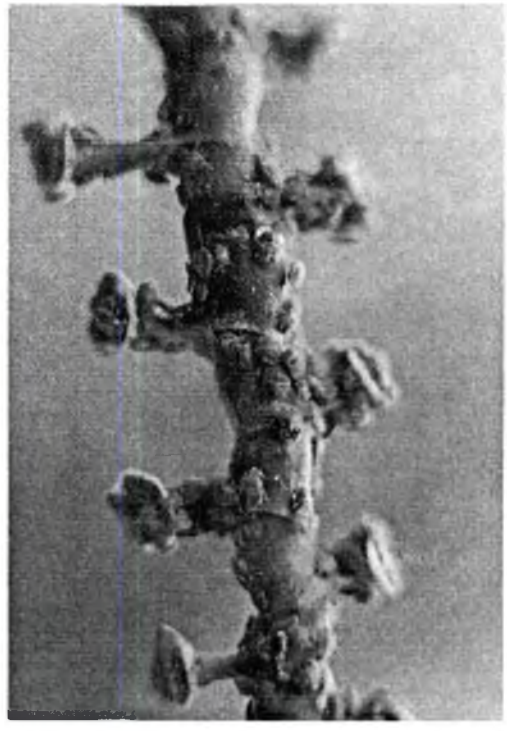

FIGURE 1. *Calamus dransfieldii* showing attack by the aphid, *Cerataphis palmae*.

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We gratefully acknowledge Prof. Raychaudhury, Calcutta University, for confirming identity of the aphid.

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A new genus *Zakaella* (Hymenoptera: Braconidae: Braconinae) from India

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ABSTRACT: A new genus *Zakaella* has been erected to accommodate *Bathyaulax alami* Zaka-ur-Rab, 1963. The genus runs close to *Iphiaulax* Foerster, but differs in having the ovipositor shorter than the metasoma with a pre-apical dorsal projection and apico-ventrally serrate, notauli distinct and well developed, and hindwing vein Ir-m very short and about half the length of SC+R1.

KEYWORDS: Hymenoptera, Braconidae, Braconinae, Iphiaulacini, *Zakaella* gen. nov., India

Zaka-ur-Rab (1963) described the species *Bathyaulax alami* based on material collected from jamboo tree, *Eugenia jambolina* Roxb, University campus, Aligarh, India. The remark Quicke (1987) about the generic placement of the species motivated the present authors to study it in detail. Types of this species got lost in Kashmir valley (Zaka-ur-Rab personal communication). The authors collected the same material from the type locality. Further study of the material reveals that *B. alami* belongs to a new genus of the tribe Iphiaulacini Telenga, except antennal flagellomeres which are less than 60 in number, a variable character of the tribe. So long, the tribe Iphiaulacini is represented by only three genera from India *viz.*, *Campyloneurus* Szepligeti, *Hybogaster* Szepligeti and *Iphiaulax* Foerster. The diagrams of generic and tribal identity along the complete description is provided in this paper. Achterberg (1993) has followed for terminologies.

***Zakaella* gen. nov. (Figs 1–9)**

Type-species: *Bathyaulax alami* Zaka-ur-Rab (1963).

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Diagnosis

Clypeus separated from the face by a well developed carnia; scapus apico-laterally strongly emarginate and distinctly so medially, longer ventrally than dorsally, terminal flagellomere acuminate and laterally compressed; mesosoma about 1.7× longer than high; notauli well developed completely; pre-coxal suture absent; metanotum and propodeum smooth; forewing vein 1-SR forms an angle of 67° with C + SC + R, vein 1-SR+M curving sharply after arising from 1-SR, vein *r* is 0.6× the length of vein m-cu, marginal cell long, SR1 reaching wing margin about 0.9× of the way between the apex of the pterostigma and the wing tip; hindwing SC+R1 about 2.0× longer than vein 1r-m; hind basitarsus 2.5× as long as hind teleotarsus; second tergite with a small well defined smooth mid-basal triangular area, antero-lateral margins depressed with a pair of posteriorly diverging sub-lateral crenulate grooves; ovipositor about 0.76× as long as metasoma with well developed pre-apical dorsal projection and apico-ventrally serrate.

Remarks

The proposed new genus *Zakaella* can be differentiated from its closely related genus *Iphiaulax* Foerster, 1862 as follows: 2nd metasomal tergite with a well defined mid-basal triangular area (In *Iphiaulax* 2nd metasomal tergite without a mid-basal triangular area), ovipositor with a pre-apical dorsal projection and apico-ventrally serrate (In *Iphiaulax* ovipositor smooth, without a pre-apical dorsal projection and apico-ventrally smooth, some times with very weak serrations only near the extreme apex), notauli distinct and well developed completely (In *Iphiaulax* notauli weakly defined anteriorly, posteriorly absolete), hindwing vein 1r-m shorter than SC+R1 (In *Iphiaulax* hindwing vein 1r-m longer than SC+R1).

Etymology

The new genus is named after Prof. Zaka-ur-Rab who described *Bathyaulax alami*, the type species of this genus.

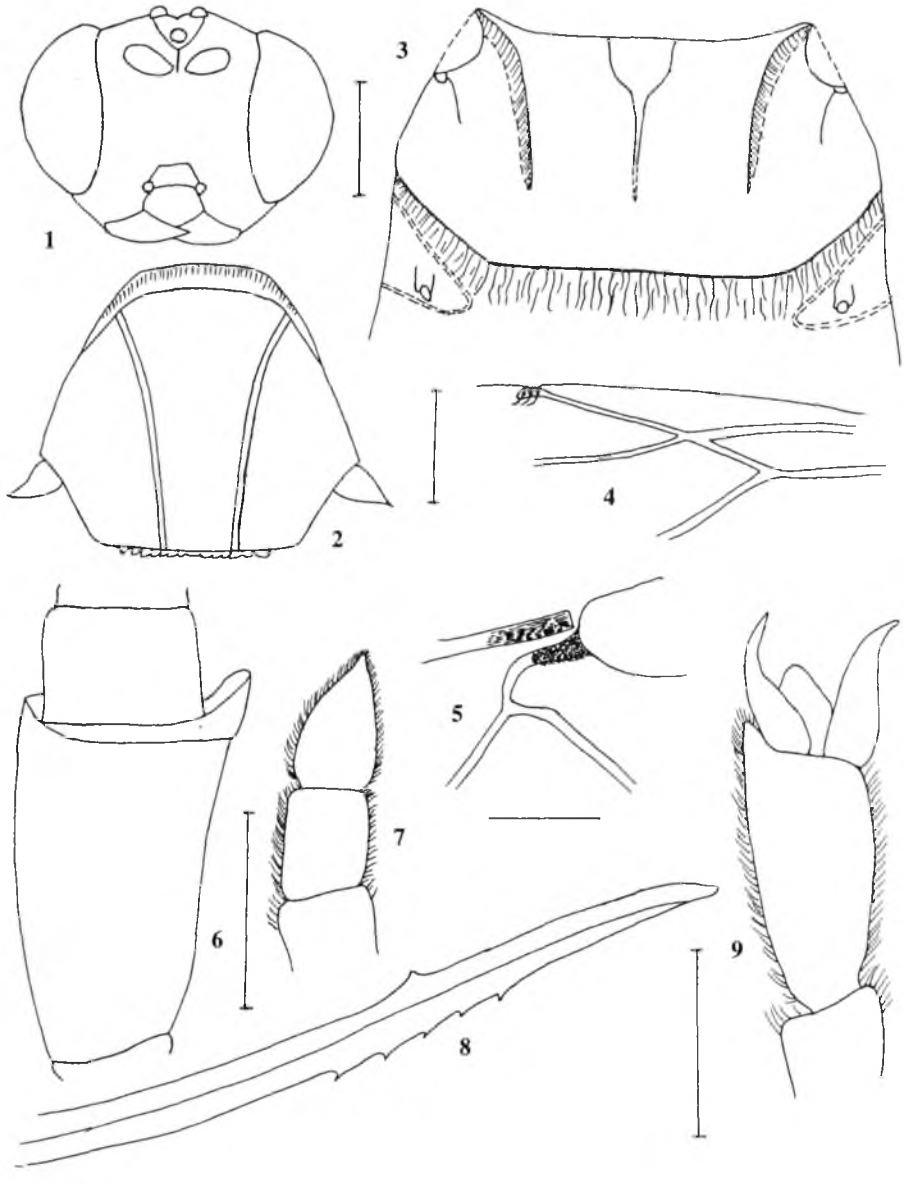
***Zakaella alami* (Zaka-ur-Rab), comb.nov.**

Bathyaulax alami Zaka-ur-Rab (1963): 192; ♀ ♂. India, Aligarh, University Campus (Type lost).

Neotype ♀, Present designation: India, Aligarh, University Campus on Jamboo tree, *Eugenia jambolina* Roxb, Catalogue No. HB-1024, 5.XI.1999, Coll. Arshad Ali Haider.

Female

Head, mesosoma, metasoma, legs and ovipositor pale orange; eye brown; tips of mandible black, ocelli transparent; proximal 1/3 of forewing pale, the rest smoky, smoky region with hyaline spots; hindwing more than half of distal uniformly smoky without any hyaline spot; body about 8 mm.



FIGURES 1–9. *Zakaella alami* gen. nov., comb. nov. 1. Head, frontal aspect. 2. Mesoscutum, dorsal aspect. 3. 2nd metasomal tergite, dorsal aspect. 4. Venation of median part of hindwing. 5. Venation of median part of forewing. 6. Scapus and pedicellus. 7. Terminal flagellomeres. 8. Apex of ovipositor. 9. Mid teleotarsus and tarsal claws. Scale lines for Figs 1–5 = 0.5 mm; 6–9 = 0.2 mm.

Head

1.25× wider than long, punctulate with hairs, without occipital carina; face about 1.5× as wide as high; malar space as long as basal width of mandible; eyes oval, about 2.0× as long as temple in dorsal view; lower margin of antennal socket distinctly raised; frons depressed with a distinct mid longitudinal carina; ocelli arranged in an equilateral triangle, stemmaticum raised, OOL : POL : AOL : ØOD = 10 : 4 : 4 : 4; mandible bidentate; antenna 47 segmented, about 0.8× the length of body and about 0.9× the forewing, scapus 1.5× longer than wide, pedicel 1.4× wider than long. F1 1.6× longer than wide, F (2-36) as long as wide, F (37-44) 1.25× longer than wide, terminal flagellomer 1.5× longer than wide; maxillary palpi five segmented; labial palpi three segmented.

Mesosoma

2.0× longer than wide; pronotum smooth dorsally with a transvers crenulate groove, projected in front of mesonotum; propleuron smooth; notauli distinct; lateral lobe of mesoscutum reticulate punctate, middle lobe anteriorly reticulate punctate, transcutlar suture complete; base of scutellar sulcus crenulate; mesopleuron smooth; metanotum smooth; metapluron smooth; forewing about 3.0× as long as wide, and nearly 1.20× longer than hindwing; vein 3-SR of forewing 2.6× longer than vein r-m, first sub-discal cell almost as long as first sub-marginal cell, first discal cell slightly shorter than first sub-marginal and first sub-discal cell, second sub marginal cell almost 1.25× as long as first sub-marginal cell, smoky region of forewing with following hyaline spots (proximal 1/3 of pterostigma, central area of first sub-marginal cell, proximal anterior angular area of second discal cell, all coalescing together to form a prominent hyaline area, an isolated oval spot in distal anterior angular area of first sub-discal cell, a small circular spot in the proximal posterior angular area of second discal cell, a linear spot along with the cross vein r-m); hindwing about 4.0× as long as wide, vein SC+R1 about 2.0× longer than 1r-m; tarsal claws simple with small basal lobe, fore tibia 0.75× as long as fore tarsus, fore tibial spur one and about 0.7× as long as fore basitarsus, fore basitarsus 1.7× as long as fore teleotarsus; mid tibia 0.85× as long as mid tarsus, mid basitarsus about 2.0×as long as mid teleotarsus; hind tibia almost as long as hind tarsus, hind basitarsus 2.5× as long as hind teleotarsus; propodeum smooth.

Metasoma

First tergite about as long as maximally wide, coriaceous with complete dorsal carina; second tergite about twice as wide as long, reticulate coriaceous, a small well defined smooth mid-basal triangular area which reaches about 0.25 of the way along the tergite, suture between 2nd and 3rd tergite crenulate; tergite 3–5 with clearly defined antero-lateral furrows defining the antero-lateral areas with well developed transverse crenulate sub-posterior grooves, tergite 3–5 granulate and 6 and 7 smooth; ovipositor shorter than metasoma, about 0.76× as long as metasoma; hypopygiun truncate, extending up to the apex of metasoma.

Male

Resembles the female except in following characters: body shorter, antennal segments variable (50–59 segmented).

Additional material examined

India, Aligarh, University Campus, 1♂, 3♀ on jamboo tree, 4.IV.2000. Coll. Arshad Ali Haider.

The neotype is deposited in the museum, Department of Zoology, Aligarh Muslim University, Aligarh 202002, India. Catalogue No. HB-1024. Additional material is in authors collection.

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Biology and feeding potential of the spider, *Oxyopes shweta* Tikader (Araneae: Oxyopidae) on lacebugs in laboratory

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ABSTRACT: Biology and feeding potential of the spider, *Oxyopes shweta* was carried out in laboratory on lacebug, *Stephanitis typicus*. Life cycle comprised of nine instars in male and ten in female. Egg to adult period last for about 95 days. A single adult consumed 8–23 lace bug per day.

KEYWORDS: Biology, feeding potential, *Oxyopes shweta*, *Stephanitis typicus*

Spiders form one of the most ubiquitous group of predaceous organisms found in any agropecosystem. Coconut agrosystem also harbours many spiders. Twenty six species of spiders belonging to twelve genera and six families were observed in association with the larval galleries of *Opisina arenosella* Walker on coconut palm (Sathiamma *et al.*, 1987). *Tetragnatha andamanensis* Tikader, *Phidippus* sp. and 21 unidentified species have been recorded as predators of the lacebug, *Stephanitis typicus* on coconut (Sathiamma *et al.*, 1998). The increasing interest on the high feeding potential of the spiders and the significant role they play in the natural suppression of lacebug, have necessitated detailed studies on their biology. Hence, an attempt is made to study the biology and feeding potential of the common hunting spider in coconut agroecosystem, *Oxyopes shweta* Tikader in laboratory on lacebug, *S. typicus* D.

Adults *O. shweta* were collected individually from lacebug infested coconut plantations at Ayiramthengu and Krishnapuram. They were reared in glass tubes (size 14 × 2.5 cm) at a temperature 28–33 °C and humidity 77 to 93% by providing *O. arenosella* larvae as prey. The eggs laid by the female spiders were kept separately and the newly emerging spiderlings were separated and kept in test tubes, provided with fresh coconut leaves colonised by known number of lacebugs. Observations were

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TABLE 1. Biology and feeding potential of *O. shweta* on lace bug, *S. typicus* in laboratory

Instar	Duration (in days)	No. of lace bugs consumed/instar
Egg + Instar I	12.5 ± 0.75	—
II	11.0 ± 0.0	12.89 ± 1.90
III	7.75 ± 1.03	20.43 ± 2.48
IV	7.0 ± 0.0	25.83 ± 2.48
V	6.0 ± 0.0	28.83 ± 3.25
VI	8.43 ± 0.53	46.50 ± 11.59
VII	8.71 ± 1.25	81.67 ± 13.56
VIII	11.17 ± 1.72	116.80 ± 16.18
IX	11.5 ± 3.15	172.70 ± 49.42
X*	15.0 ± 0.89	289.4 ± 21.49

* Only female has Xth instar.

made daily on the duration of different life stages, moulting, longevity of adults and feeding potential of immature stages as well as adults.

O. shweta is found in field in abundance during June–September with a peak in July. The spider does not make web but pounces on the prey. Five to ten days after mating, the female lays the first egg sac (cocoon) inside the test tube. Egg sac is white and centicular. 3 to 4 such egg sacs are constructed by a single female. The number of eggs per cocoon varies from 16 to 82 with maximum number in the first cocoon to minimum in the succeeding ones. The total number of eggs laid by a female averaged 159.

Life cycle of *O. shweta* comprises nine instars in male and ten instars in female. First instar is inside the egg sac. Egg stage and first instar last for 12 to 13 days. Newly hatched second instar emerges out from egg sac and feed on lacebugs. Duration of different instars and number of lacebugs consumed/instar are given in Table 1. During second instar it consumed 12.89 ± 1.9 lacebugs. Thereafter, a steady increase in prey consumption occurs reaching a maximum of 172.70 ± 49.43 in males and 289 ± 21.49 in females. Thus the rate of predation showed an eight fold increase and fourteen fold increase in male and female from second to ninth instars and second to tenth instars, respectively. Egg to adult period last for about 95 days. A single adult spider consumed 8–23 lace bugs per day in laboratory.

O. shweta feeds on a variety of insects including Thysanoptera, Orthoptera, Homoptera, Diptera, Coleoptera and Lepidoptera. It feeds very freely on Leipdopteran larvae such as cotton boll worms and leaf eating caterpillars. It has been recorded as a common spider in sugarcane and coconut agroecosystem in India (Easwaramoorthy *et al.*, 1996; Nair *et al.*, 2000). *O. shweta* is a well known predator on the sucking pests of sugarcane *viz.*, the aphid, *Melanaphis indosacchari David*, the leaf hopper, *Pyrilla perpusilla* Walker and the pink melay bug, *Saccharicoccus sacchari* (Ckll). It also accepted readily larvae of Chilo infuscatellus, Chilo sacchariphagus *indicus* Kapur

and *Sesamia inferens* Walker. Oxyopidae is the most common spider family collected from cotton, okra, groundnut, sugarcane, cabbage and rice ecosystem in India (Peter and Biswas, 1990).

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Argyrodes flagellum (Araneae: Theridiidae), a rare comb-footed whip spider: First report from India

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ABSTRACT: *Argyrodes flagellum*, a rare comb-footed whip-spider so far known only from Singapore, was recorded for the first time from India at Ernakulam District in Kerala.

KEYWORDS: *Argyrodes flagellum*, whip spider Theridiidae, first report

Spiders of the genus Argyrodes Simon of the Family Theridiidae are little known from the Indian region. Simon (1864) was the first to report some species of this genus. Koch (1872); Thorell (1890); Exline and Levi (1962) were the scientists who worked on this genus. Later Tikader (1970, 1977) reported seven species of this genus from Sikkim and Andaman-Nicobar Islands of India. Koh (1989) reported this species for the first time from Singapore.

Recently two female specimens of *Argyrodes flagellum* were collected from a fencing in Varapuzha, Ernakulam District, Kerala (Collector: A. V. Sudhikumar on 23 December 2000) and a male specimen from Kakkanad, Ernakulam District, Kerala (Collector: Samson Davis on 11 November 2001). It is a rare species of spider and has some interesting characters that need to be described, since it has not been reported from India so far. Outside India, this species was reported only from Singapore.

Description (Fig. 1)

Female: Total length 19.1 mm, cephalothorax 2.9 mm long, 1.14 mm wide. Cephalothorax dark green in colour with a mid-dorsal longitudinal yellow band. Sub posterior of the cephalic area higher than the thoracic area. Eight eyes in two rows. Anterior row of eyes recurved, posterior row slightly procurved. Eye diameter (mm): AME = 0.071, PME = 0.034, ALE = 0.042, PLE = 0.033. Eye separation: AME–AME = 0.077, AME–ALE = 0.040, PME–PME = 0.105, PME–PLE = 0.038. AME twice times larger than PME. Posterior side of ocular

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TABLE 1. Length of leg segments of the spider *Argyrodes flagellum* (mm)

	Length of leg segments (mm)											
	Femur		Patella		Tibia		Metatarsus		Tarsus		Total	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
I	6.12	5.05	0.38	0.29	2.04	1.94	6.02	4.95	1.45	1.36	16.01	13.59
II	3.20	2.72	0.29	0.38	1.94	1.74	2.23	2.04	1.06	0.97	08.72	07.85
III	1.55	1.36	0.19	0.19	0.68	0.87	1.06	0.97	0.77	0.58	04.25	03.97
IV	6.22	4.76	0.48	0.38	4.08	3.21	5.15	4.01	1.74	1.45	17.67	13.81

quadrangle wider than anterior side. Ocular group hexagonal in shape. Lateral eyes contiguous. Sternum longer than wide. Pointed posterior end with dark green in colour. Maxillae yellow, longer than wide, subbassaly broad. Chelicerae small, brown with 2 teeth on pro-margin. Legs slender and long. Fourth leg very long. Green in colour with yellow band in femora, tibia and metatarsi of all legs, with out strong spines. Seven serrated bristles are present on the fourth tarsus. Leg formula 4/1/2/3. Length of leg segments is given in Table 1.

Abdomen 16.2 mm long, 1.11 mm wide, light green, long and tapering to a point posteriorly with silver-white scales along the sides. Five times longer than cephalothorax. Epigyne situated near the anterior end. Spinnerets are about one fifth of the distance from the anterior end. Epigyne encircled by black hairs. Epigyne and internal genitalia as in Fig. (*f*) and (*g*). Black strong hairs are projecting towards the cephalothorax from the anterior end of abdomen.

Male: Total length 12.15 mm, cephalothorax 1.16 mm long, 1.09 mm wide. Ocular group hexagonal with ocular region slightly curved down ward. Eye diameter (mm): AME = 0.068, PME = 0.032, ALE = 0.039, PLE = 0.032. Eye separation: AME–AME = 0.072, AME–ALE = 0.039, PME–PME = 0.108, PME–PLE = 0.036. Legs are green in colour with yellow band present in the femora and metatarsi only. Serrated bristles are absent on the fourth tarsus. Leg formula 4/1/2/3. Length of leg segments is given in Table 1.

Abdomen 10.99 mm long, 1.01 mm wide. Shape of abdomen same as female. Pedipalp brownish except yellow bands in the swollen sub apex of femur, apex of patella and mid half of tibia. Embolus with a slightly transparent, enlarged and open tip. Structure of pedipalp as in Fig. (*h*).

Natural history

Two females were collected from a pepper plant, one with a white cocoon and one male was collected from a coconut plantation. This strange spider looks like a bit of tendril. While resting, it lines up its tiny thin legs to form a straight line. The small, superbly camouflaged spider is hard to spot. Only when it moves does it become obvious. This whip spider does not make a web and instead hangs on a simple Y-shaped silken trap. It may seem impossible for an insect to get snagged onto a single line of silk. By appearing like an extension of a tendril, the spider may appear to be

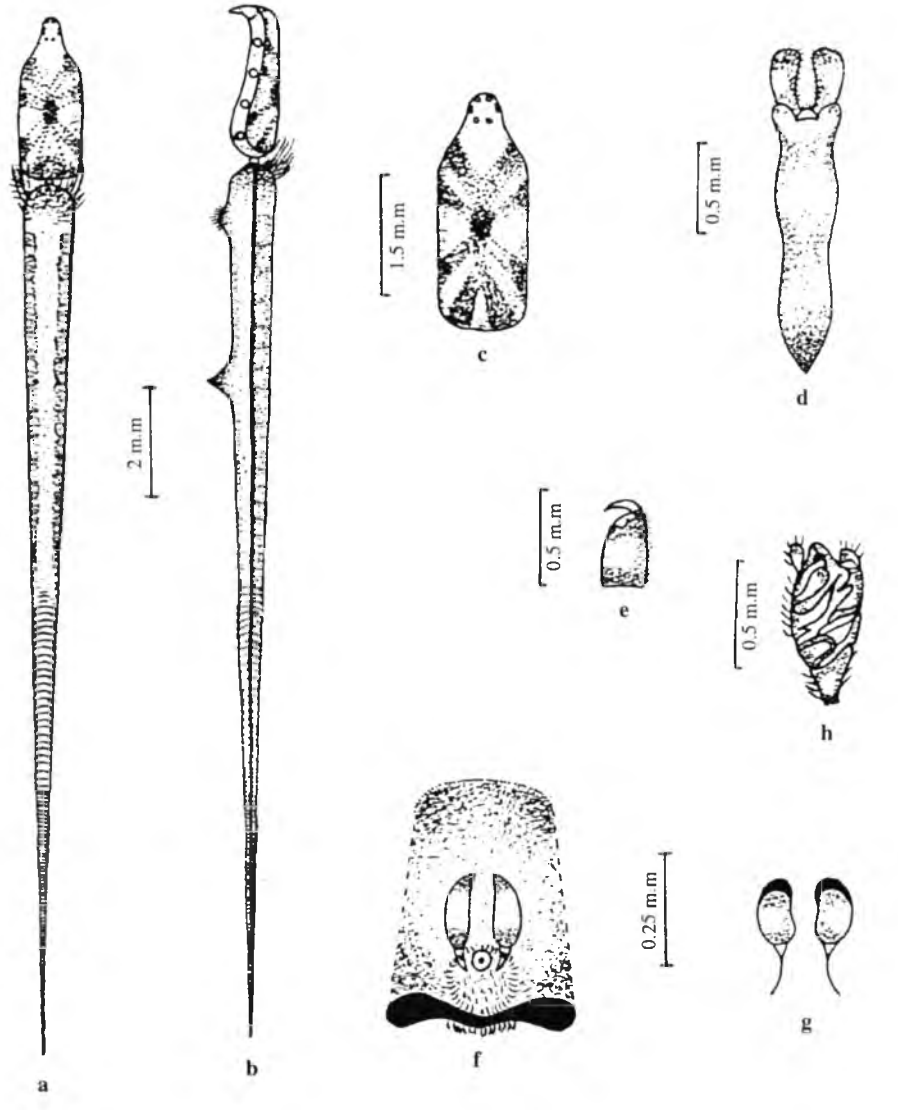

FIGURE 1. *Argyrodes flagellum*: a. Female dorsal view; b. Lateral view; c. Cephalothorax; d. Sternum with Labium and Maxillae; e. Chelicera; f. Epigyne; g. Internal genitalia; h. Pedipalp.

a safe perch for small unsuspecting flying insects. The specimens are deposited at the Division of Arachnology, Department of Zoology, Sacred Heart College, Kochi-13.

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Laboratory assessment of the potentiation of neem extract with the extracts of sweet-flag and pungam on bhendi shoot and fruit borer, *Earias vitella* (Fab.)

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ABSTRACT: Potentiation of neem extract (*Azadirachta indica*) (N) with extracts of sweet-flag (*Acorus calamus*) (S) and pungam (*Pongamia glabra*) (P) at 1 : 1 : 1 (NSP I), 2 : 1 : 1 (NSP II) and 3 : 1 : 1 (NSP III) (v/v) ratios for the control of *Earias vitella* was studied in the laboratory. The mixtures were more effective. The mortality of shoot and fruit borer was maximum (93.33 per cent) in NSP (I) compared to neem alone. NSP (I) recorded an EC_{50} (Effective concentration) of 0.1180% and LT_{50} (Lethal time) value of 96.66 h against the third instar larvae of *E. vitella*. Deformities in larvae, pupae and adults of *E. vitella* were also more in combination treatments compared to neem treatment.

KEYWORDS: Neem, sweet-flag, Pungam, *Earias vitella*, botanical mixture

Among the phytochemical sources, neem has been used since time immemorial by Indian farmers and its pest controlling properties were scientifically documented in laboratory and field tests. Neem extracts and its pure compounds were reported to cause mortality when eaten along with food (Rembold *et al.*, 1981; Meisner *et al.*, 1986) or applied topically (Reguraman and Saxena, 1994) on many crop pests. Toxicity of sweet-flag (Nair and Thomas, 2000) and pungam (Jayaraj and Regupathy, 1999) extracts was also well documented against various insect pests. Although neem shows effective pest controlling properties, its lower toxicity and faster photo-degradability limit its use by farmers and its toxicity against insect pests is known to be improved when combined with other botanicals (Ganeshan *et al.*, 1995; Nelson, 1996). An attempt was made to evaluate the joint action potential of neem in combination with other botanical extracts against shoot and fruit borer, *E. vitella*.

Seed kernels of neem and pungam and rhizomes of sweet-flag were ground to fine powder and their extracts were obtained in methanol by cold extraction. The extracts

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were made free of methanol in distillation unit at 50 °C under reduced pressure. The following botanical mixtures were prepared.

1. Neem + Sweet-flag + Pungam in 1 : 1 : 1 (v/v) (NSP I),
2. Neem + Sweet-flag + Pungam in 2 : 1 : 1 (v/v) (NSP II) and
3. Neem + Sweet-flag + Pungam in 3 : 1 : 1 (v/v) (NSP III) and formulated to 60 EC using an organic solvent (Cyclohexanone) and an emulsifier (Unitox®).

Neonate larvae obtained from sterilized eggs were transferred individually using soft camel hairbrush on to the bhendi fruit disc-pieces kept in multi-cavity tray. The fruit disc-pieces were replaced daily until pupation. The required number of larvae were taken from this culture for conducting the experiments upon hatching. Bhendi-fruit disc-pieces dipped in test concentrations for 30 seconds were shade dried, placed in plastic cups. Batches of 20 pre-starved (3 h) third instar larvae were released into each treatment. There were 9 treatments each replicated thrice. Larvae released on solvent treated fruit disc-pieces served as control. After allowing 48 h for feeding, the larvae in all the treatments were provided with untreated fresh fruit disc-pieces and were continued till the death of larvae or pupation. The number of larvae pupated and number of adults emerged were recorded. The effective concentration (EC_{50}) and lethal time (LT_{50}) values were estimated by probit analysis (Finney, 1971) and rest of the data were subjected to analysis of variance (ANOVA).

Among the treatments, NSP (I) @0.3% recorded a maximum of 93.33 per cent mortality on 10 DAT followed by NSP (II) and (III) @0.3% with 83.33 and 76.67 per cent mortality, respectively (Table I). Neem @0.3% inflicted 73.33 per cent mortality on 10 DAT. The per cent pupation and adult emergence were drastically reduced in all the treatments compared to control. The per cent pupation and adult emergence were up to 14.16 and 6.6 in botanical mixture treatment, while they ranged from 9.16 to 22.50 and 5.0 to 18.33, respectively, in treatment with neem alone. The larval period was prolonged more significantly in all the treatments compared to control.

A prolongation of larval duration (upto 5 days) was observed in all the botanical treatments compared to control, particularly at lower concentration (Table 1). This was more pronounced in neem individual treatment than botanical mixtures. The reason might be that at higher concentrations of botanical mixtures, their higher toxicity would kill the larvae rather than exhibiting IGR activity. In other words, the botanical mixtures showed increased toxicity and reduced IGR activity. Lot of evidence is available on the IGR activity of neem, where treatment with neem products (Azadirachtin) caused physiological effects like reduction, delay or absence of ecdysones and juvenile hormones during the last larval instars and nymphal periods (Rembold *et al.*, 1987). Azadirachtin probably has more than one sites of action, depending on the time and growth stage of test insects (Koul and Isman, 1991). Several larvae displayed morphological deformities, bloated abdomen and larva-pupa intermediates (Haubruge *et al.*, 1994). In the present study, during the development, larvae also lost their body weight rapidly due to excessive defecation and feeding inhibition and transformed into small size and shrivelled pupae and in many cases larvae were unable to produce silken cocoons and transformed into naked pupae.

TABLE 1. Toxicity of botanical mixtures to third instar larvae of *E. vitella*

Treatment	Per cent corrected mortality (10 DAT)*	Per cent pupation	Per cent adult emergence	Larval period (days) (DAT)
Neem 0.18%	56.67	22.50	18.33	15.28
	(48.84)^{h}	(28.29)^{e}	(25.34)^{d}	
Neem 0.3%	73.33	9.16	5.00	14.83
	(59.00)^{d}	(17.58)^{c}	(12.63)^{c}	
NSP (I) 0.18%	70.00	8.23	1.66	14.61
	(56.79)^{e}	(16.74)^{c}	(6.23)^{b}	
NSP (I) 0.3%	93.33	0.00	0.00	13.24
	(77.53)^{a}	(0.52)^{a}	(0.52)^{a}	
NSP (II) 0.18%	66.67	13.33	5.83	14.72
	(54.78)^{g}	(21.39)^{d}	(13.91)^{c}	
NSP (II) 0.3%	83.33	0.00	0.00	14.00
	(66.14)^{b}	(0.52)^{a}	(0.52)^{a}	
NSP (III) 0.18%	66.67	14.16	6.66	14.80
	(55.07)^{f}	(22.09)^{d}	(14.90)^{c}	
NSP (III) 0.3%	76.67	3.15	0.00	14.28
	(61.22)^{c}	(10.03)^{b}	(0.52)^{a}	
Control (Solvent)	—	82.50	78.33	10.55
	—	(65.30)^{f}	(62.26)^{e}	

*Mean of three replications. Figures in parentheses are arcsine transformed values. Means in a column followed by same letter are not significantly different ($p = 0.05$) by DMRT.

TABLE 2. LT_{50} and EC_{50} values of botanical mixtures against third instar larvae of *E. vitella*

Treatment	LT_{50} (H)	Fiducial limits		Regression equation	Relative activity
		Lower	Upper		
Neem 0.18%	217.19	188.97	248.65	$Y = -5.3476 + 4.4280X$	1.000
Neem 0.3%	160.28	179.47	142.46	$Y = -3.7119 + 3.9511X$	1.000
NSP (I) 0.18%	150.48	142.46	179.47	$Y = -1.0291 + 2.2571X$	1.443
NSP (I) 0.3%	96.66	76.35	114.60	$Y = 0.5499 + 2.2571X$	1.711
NSP (II) 0.18%	178.90	159.95	199.52	$Y = -5.0589 + 4.4654X$	1.214
NSP (II) 0.3%	111.78	95.94	130.01	$Y = -0.9224 + 2.8912X$	1.433
NSP (III) 0.18%	182.93	158.78	210.47	$Y = -2.9805 + 3.5276X$	1.181
NSP (III) 0.3%	128.64	109.90	149.96	$Y = -0.8615 + 2.7872X$	1.245
	EC_{50} (%)				
Neem	0.1798	0.1793	0.1804	$Y = -7.1753 + 3.7402X$	1.000
NSP (I)	0.1180	0.1018	0.1361	$Y = -5.3081 + 3.3553X$	1.524
NSP (II)	0.1336	0.1155	0.1539	$Y = -5.3658 + 3.3161X$	1.346
NSP (III)	0.1378	0.1185	0.1599	$Y = -4.5610 + 3.0457X$	1.305

In the present study, NSP (I) recorded EC_{50} of 0.1180% followed by NSP (II) and NSP (III) with 0.1336 and 0.1378%, respectively, whereas neem had EC_{50} value of 0.1798% (Table 2). The relative activity of botanical mixtures tested for EC_{50} increased from 0.305 to 0.524 fold over the neem treatment. Lethal time (LT_{50}) values were short (96.66 h) in NSP (I) @0.3% followed by 111.78 h in NSP (II) @0.3% and 128.64 h in NSP (III) @0.3% treatment. However, neem treatment recorded the LT_{50} of 160.28 h and 217.19 h at 0.3 and 0.18% concentrations, respectively. The relative activity of botanical mixtures tested for LT_{50} values increased from 0.181 to 0.711 fold over the neem treatment (Table 2). Here, the data on the effect of botanical combination (NSP) indicated a significant increased mortality to larvae of *E. vitella* over the treatment of neem. This is in line with the findings of Ganeshan *et al.* (1995) and Nelson (1996) who reported that larval mortality of *S. litura* and *E. vitella* were more when different plant products were combined, than when applied individually. This may be due to joint action of different toxic principles in mixture that influenced different sites of action physiologically and biochemically resulting in quicker mortality. Reduced feeding observed in the present study may also play an important role in the increased mortality.

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